Universidade de Aveiro Departamento de Biologia
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Comunidades macrobentónicas dos vulcões de lama do Golfo de Cádis

Macrofaunal assemblages from mud volcanoes in the Gulf of Cadiz

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica da Professora Doutora Maria Marina Ribeiro Pais da Cunha, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro.

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À memória da minha tia-avó Leonor Nogueira

o júri/ the jury

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palavras-chave Macrofauna, espécies quimiossintéticas, relações tróficas, endossimbiontes, vulcões de lama

resumo

Desde a descoberta em 1999 do primeiro vulcão de lama no Golfo de Cádis, cerca de 40 locais, de profundidade variável entre os 200 e os 3900 m, com diferentes graus de emissão de hidrocarbonetos foram localizados e amostrados dentro do programa IOC-UNESCO "Training Through Research (TTR) " e mais recentemente dentro do projecto europeu HERMES.

Neste estudo investigamos as comunidades da macrofauna dos vulcões de lama do Golfo de Cádis utilizando uma diversidade de equipamento de amostragem quantitativo e não quantitativo. Mais de 14550 espécimes foram examinados e incluídos nos diferentes grupos taxonómicos, sendo fornecida uma lista taxonómica detalhada com o menor nível taxonómico possível. A biodiversidade, distribuição dos principais taxa, as espécies quimiossintéticas e a biodiversidade regional e substituição de espécies são apresentados e discutidos.

Dentro da macrofauna, os bivalves (nomeadamente super-familia Thyasiroidea, espécies quimisimbióticos e comunidade de bivalves) e os ofiurideos são estudados em pormenor.

Os Thyasiroidea colhidos nos vulcões de lama do Golfo de Cádis são revistos. Das sete espécies identificadas, apenas uma *Thyasira vulcolutre*. sp. nov se encontra associada a um ambiente quimiossintético. Esta espécie é restrita a locais activos, mas não se verificam padrões de distribuição para as outras espécies.

Os bivalves quimiosimbióticos amostrados são revistos. Das 10 espécies fortemente associadas a ambientes quimiossintéticos duas Solemyidae, *Petrasma elarraichensis* sp. nov. e *Acharax gadirae* sp. nov., uma Lucinidae, *Lucinoma asapheus* sp. nov., e uma Vesicomyidae, *Isorropodon megadesmus* sp. nov. são descritas e comparadas com similares das respectivas famílias. As comunidades de bivalves foram analisadas em detalhe e do estudo de 759 espécimes (49 espécies em 21 familias) descreve-se a diversidade e padrões de distribuição.

Os Ophiuroidea amostrados nos vulcões de lama e ambientes batiais adjacentes são revistos. Treze espécies são incluídas em 4 famílias, Ophiacanthidae, Ophiactidae, Amphiuridae e Ophiuridae e são identificadas, tendo sido descrita uma nova espécie *Ophiopristis cadiza* sp. nov.

Rácios isotópicos ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) foram determinados em várias espécies no intuito de investigar a ecologia trófica das comunidades bênticas dos vulcões do Golfo de Cádis. Os valores de $\delta^{13}C$ para os bivalves Solemyidae, Lucinidae e Thyasiridae estão de acordo com os valores para outros bivalves conhecidos por possuírem simbiontes tiotróficos. Por outro lado os valores de $\delta^{13}C$ e $\delta^{34}S$ para *Bathymodiolus mauritanicus* sugerem a ocorrência de metanotrofia. A análise da fauna heterotrófica indica igualmente que as espécies habitantes da cratera dos vulcões de lama derivam a sua nutrição de fontes quimiossintéticas.

resumo (cont.)

A indicação pela análise isotópica que as bactérias autotróficas contribuem substancialmente para a nutrição dos bivalves hospedeiros, levou-nos a investigar os endossimbiontes e as suas relações filogenéticas relativamente a outros bivalves através da análise comparativa de análises de sequências de 16S ribossomal RNS. Análises moleculares PCR-DGGE (Denaturing Gradient Gel Electrophoresis) e clonagem de genes de bacterias 16S rRNA confirmaram a presença de simbiontes oxidantes de enxofre e colocam a possibilidade de uma simbiose dupla para o *B. mauritanicus*. A diversidade microbiana dentro dos Frenulata foi igualmente estudada recorrendo a métodos moleculares e revelou a não existência de padrão entre espécies, vulcões, profundidade e idade do animal sugerindo assim a não procura de simbiontes específicos.

keywords Macrofauna, chemosynthetic species, trophic relationships, endosymbionts, mud volcanoes

abstract

Since the discovery in 1999 of the first mud volcano in the Gulf of Cadiz, about 40 other sites at depths ranging from 200 to 3900m, with varying degrees of hydrocarbon-rich gas seepage activity, have been located and sampled under the IOC-UNESCO Training Through Research (TTR) programme and, more recently, the EU funded HERMES project.

In this study we investigate the macrofaunal assemblages from mud volcanoes in the Gulf of Cadiz using a diverse array of quantitative and non-quantitative sampling gear. More than 14550 specimens were examined and ascribed to different taxonomic groups and a detailed taxonomic list with the lowest possible taxonomic level possible at the moment is provided. The biodiversity and distribution of major taxa, the chemosymbiotic species and the regional biodiversity and species turnover are present and discussed.

Within macrofauna, bivalves (namely the super-family Thyasiroidea, chemosymbiotic species and bivalves assemblages) and ophiuroids were studied in detail.

Thyasiroidea collected from the mud volcanoes of the Gulf of Cadiz are reviewed. Of the seven species identified only one, *Thyasira vulcolutre*. sp. nov, is closely associated with a chemosynthetic setting. *Thyasira vulcolutre* is restricted to active seeps but there is no pattern in the distribution of the other thyasirid species.

The chemosymbiotic bivalves collected are reviewed. Of the ten species closely associated with chemosynthetic settings two Solemyidae, *Petrasma elarraichensis* sp. nov. and *Acharax gadirae* sp. nov., one Lucinidae, *Lucinoma asapheus* sp. nov., and one Vesicomyidae, *Isorropodon megadesmus* sp. nov. are described and compared to close relatives of their respective families.

The deep-water bivalve assemblages were also analyzed in detail. From the study of 759 specimens representing 49 species in 21 families, the diversity, distribution patterns and species turnover are described.

The Ophiuroidea collected from mud volcanoes and adjacent bathyal environments from the Gulf of Cadiz are reviewed. Thirteen species included in four families, Ophiacanthidae, Ophiactidae, Amphiuridae and Ophiuridae were identified and one new species *Ophiopristis cadiz*a sp. nov was described.

Stable isotopes ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) were determined in several species to investigate the trophic ecology of the benthic assemblages from mud volcanoes in the Gulf of Cadiz. The $\delta^{13}C$ values for solemyid, lucinid and thyasirid bivalves are in line with data for other bivalves known to host thiotrophic symbionts. On the other hand, $\delta^{13}C$ and $\delta^{34}S$ values for the bathymodiolid species are compatible with the predominance of methanotrophy.

abstract (cont.)

The analyses of heterotrophic fauna indicate that the species inhabiting the crater of the mud volcanoes derive the bulk of their nutrition from chemoautolithotrophic sources.
The indication by stable isotope analysis that autotrophic bacteria make a substantial contribution to the nutrition of the bivalve hosts, led us to investigate the bacterial endosymbionts and their phylogenetic relationship to other symbionts in bivalve hosts based on comparative 16S ribosomal RNA gene sequence analysis. Molecular analysis using PCR-DGGE (Denaturing Gradient Gel Electrophoresis) and cloning of bacterial 16S rRNA genes confirmed the presence of sulphur-oxidizing symbionts within gill tissues of the studied bivalve specimens and point out to the possibility of a dual sumbioses for *Bathymodiolus mauritanicus*
The microbial diversity inside frenulata worms was also analyzed using the same molecular tools and revealed no patterns between species, MV, depth, age of animal and therefore seems to suggest that they do not seem to select for specific symbionts.

Reino de medusas e água lisa
Reino de silêncio luz e pedra
Habitação das formas espantosas
Coluna de sal e círculo de luz
Medida da Balança misteriosa

In “Mar” from Sophia de Mello Breyner

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SECTION 1. INTRODUCTION

Chapter 1.1.Introduction to the cold seeps

The deep-sea, which covers more than 70% of earth's surface, is generally nutrient-poor, devoid of sunlight, and imposes extreme conditions including high pressure and low temperatures. In the late seventies, complex biotic systems were discovered at deep-sea hydrothermal vents (Corliss & Ballard 1977; Lonsdale 1977; Corliss *et al.* 1979). These ecosystems do not depend on sunlight as the primary energy source, but instead derive energy from chemicals in a process named chemosynthesis or chemotrophy (Cavanaugh *et al*. 1981). The existence of such chemotrophy-based systems has been a revelation with respect to our view of the beginning of life and ecosystem functioning in our planet (Heijs 2005). Later cold seeps (Kennicutt *et al*. 1985; Suess *et al*. 1985; Kulm *et al*. 1986; Le Pichon *et al*. 1987) and large organic falls (Deming *et al.* 1997; Smith & Baco 2003) have also been identified as chemotrophy-based environments.

Cold seeps are areas on the ocean floor where water, minerals, hydrogen sulphide, methane, other hydrocarbon-rich fluids, gases and muds are leaking or expelled through sediment layers and faults by gravitacional forces and/or overpressures in often gas-rich subsurface zones (Figure 1.1.1). In contrast to hydrothermal vents, these emissions are not geothermally heated and therefore much cooler, often close to surrounding seawater temperature. In cold seeps areas, both methane and sulphide are available in high concentrations and support large biomasses of tube worms, mussels, vesicomyid clams and giant sulphide-oxidizing bacteria (MacDonald *et al*. 1989; Fisher 1990; Sibuet & Olu 1998; Sahling *et al*. 2002), in many aspects similar to those found at hydrothermal vents. These communities are often associated with authigenic carbonates building chemoherms (Bohrmann *et al*. 1998, 2002) features which resemble reefs and/or chimneys (Hovland & Judd 1988; Roberts & Aharon 1994; Michaelis *et al*. 2002).

Cold seeps were first found in the deep ocean during dives in the Florida Escarpment, Gulf of Mexico (Paull *et al.* 1984; Kennicutt *et al*. 1985) and in subduction zones of the NE Pacific (Suess *et al*. 1985; Kulm *et al*. 1986) and NW Pacific (Le Pichon *et al*. 1987). Following these first discoveries and the rapid succession of both modern and fossil cold seeps' records, it was clear

that these reducing environments are geologically diverse and widespread (Figure 1.1.2), occurring in all continental margin environments at virtually all depths (Sibuet & Olu 1998; Tunnicliffe *et al.* 2003; Levin 2005).

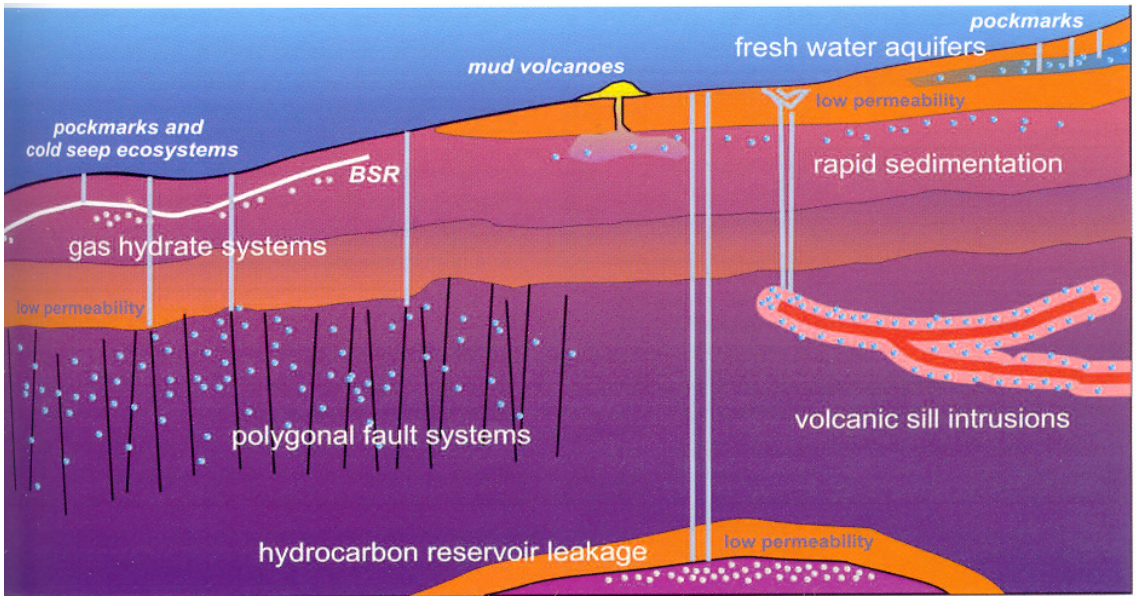

Figure 1.1.1. Schematic representation of cold seeps and other focused fluid flow systems/features. From Berndt (2005).

Most studies on cold seeps report from North Pacific and North-western Atlantic (e.g. Gulf of Mexico: MacDonald *et al.* 1994; Bergquist *et al*. 2003: Barbados: Olu *et al*. 1996; Barents Sea: Egorov *et al*. 1999; Cascadia margin: Suess *et al*. 1999; Sahling *et al*. 2002). It was only during the past decade that more intense investigation of the European continental margins using in situ video and photography with adapted deep submersibles, provided evidence for a wide range of active cold-seep ecosystems associated with fluid escape structures. These structures include pockmarks, brine lakes, and elevated or flat mud volcanoes (Vanreusel *et al.* 2009). Along the European margins cold seepage is known from the North Sea and Norwegian margin in the Kattegat (Dando *et al.* 1991; Jensen *et al*. 1992), in the Skagerrak (Dando *et al.* 1994) and the Haakon Mosby (Milkov *et al.* 1999; Pimenov *et al.* 1999); from the eastern Mediterranean (Corseli & Basso 1996; Salas & Woodside 2002; Olu-Le Roy *et al.* 2004) where intense emission of methane occurs from the center of active mud volcanoes and along related faults in the Calabrian Arc and Mediterranean Ridge and Anaximander Mountains (Vanreusel *et al.*

2009), and from the Western Mediterranean in the Alboran Sea (Maldonado & Comas 1992). Since their initial discovery in the Gulf of Cadiz in 1999 (Kenyon *et al.* 2000; Gardner 2001) a large number of mud volcanoes have been identified in this region. Located in four main fields, the mud volcanoes from the Gulf of Cadiz exhibit different but generally very localized hydrocarbon seepage (Niemman *et al.* 2006).

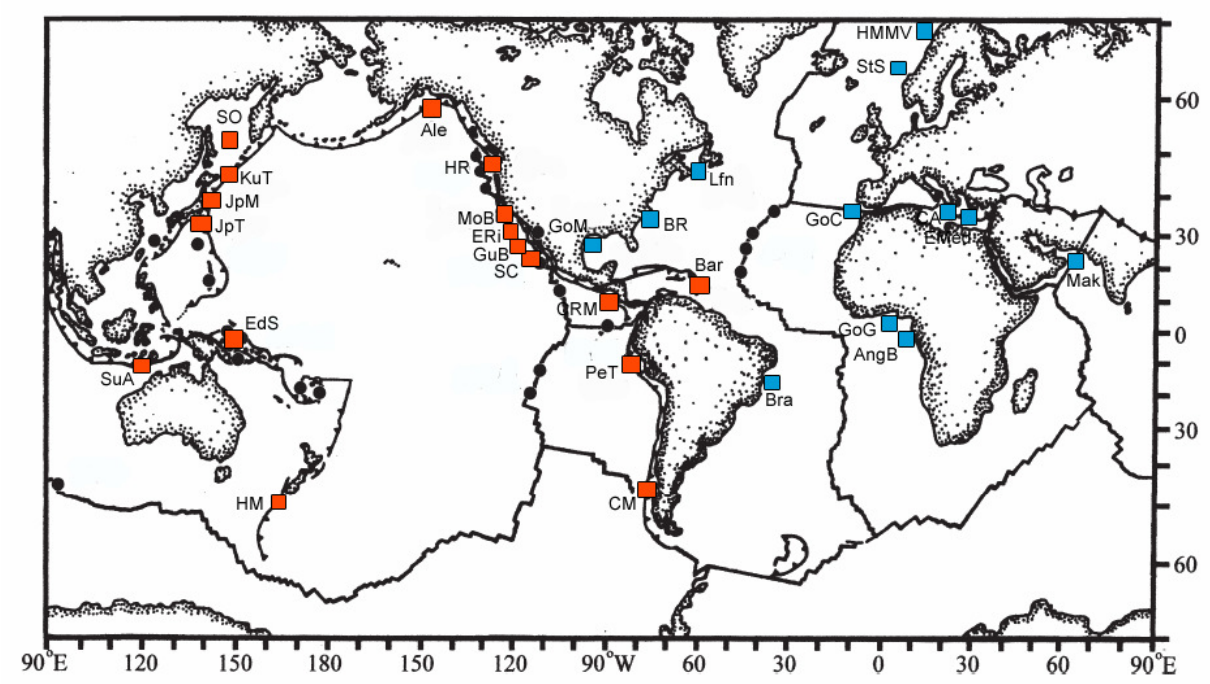

Figure 1.1.2. Worlwide distribution of cold seeps. Sites in red indicate active margins, sites in blue lie on passive margins although the classification of the Gulf of Cadiz and the Mediterranean Ridge as passive margins is debatable. **Ale**: Aleutian Margin/Trench, **AngB**: Angola Basin, **Bar**: Barbados, **BR**: Blake ridge, **Bra**: Brasil, **CM**: Chile Margin, **CA**: Calabrian Arc, **CRM**: Costa Rica Margin, **EdS**: Edison Seamount, **ERi**: Eel River, **GoC**: Gulf of Cadiz, **GoG**: Gulf of Guinea, **GoM**: Gulf of Mexico, **GuB**: Guyama Basin, **HM**: Hikurangi Margin (New Zealand), **HMMV**: Haakon Mosby Mud volcano, **HR**: Hydrate Ridge, **JpM**: Japan Margin, **JpT**: Japan Trench, **KuT**: Kurile Trench, **Lfn**: Laurentian fan, **Mak**: Makran, Pakistan, **EMed**: Numerous sites on Eastern Mediterranean Sea, **MoB**: Monterey Bay, **PeT**: Peru Trench, **SC**: Southern California, **SO**: Sea of Okhotsk, **StS**: Storegga Slide, **SuA**: Sunda Arc. Adapted from Tunnicliffe *et al.* (2003).

The source of methane-rich fluid may be a few kilometers below surface sediments (Figure 1.1.1). Subsurface fluid migration has many implications for the fluid flow and the associated fluid chemistry at seeps. Fluids migrate preferably along more permeable pathways such as coarse-grain strata and tectonically induced faults and fractures. Consequently fluid velocities are 3 to 4 orders of magnitude faster under focused flow conditions, e.g. along faults as compared to diffuse flow conditions. Diffuse flow refers to fluid transport in a rather homogeneous porous media and does not relate to (molecular) diffusion. Fluid transport is not only vertical; significant lateral fluid transport also occurs before soft sediment is reached. In contrast, in little compacted surface sediment only upward-directed transport can be expected. Fluid advection is induced if a pressure gradient greater than the ambient hydrostats pressure gradient exists between the surface and the sediment surface. Causes of increased pressure at depth may be manifold and include compression through plate movements, gas formation, and fluid-rock interactions, leading to a release of crystal-bound water into ambient pore fluid (Ziebes & Haese 2005).

Pore water concentrations profiles from seep and non-seep environments are distinct due to differences in the solute transport regime. In sediments that are not affected by fluid advection, no concentration gradients occur for conservative solutes such as chloride (Figure 1.1.3). In contrast, in seep sediments the advected fluid and the ocean (bottom) water mix, which is reflected in changes in the concentration with depth of an inert solute, if the fluid chemistry between the two water sources differs.

Cold seeps are often associated with gas hydrates, and the potential of methane expelled has been considered of extreme importance in the context of carbon cycling in the continental margins. Gas hydrates are solid ice-like structures consisting of gas (predominantly methane) which is encaged inside ice crystals. Gas hydrates form under conditions of low temperature, high hydrostatic pressure, and gas in excess of solubility (Kvenvolven 1988; Buffet 2000). Hydrocarbons are the second most important fluid on Earth; methane is the lightest and simplest form of hydrocarbon and it is probably the most abundant and widespread of all the gases held within the sedimentary rocks of

the crust (Hedberg 1980). Methane is released from subsurface reservoirs and ascends as free gas or dissolved in fluids to the sediment surface (Figure 1.1.4). Methane can be formed by thermogenic degradation of organic material buried in the sedimentary column or by biogenic consumption of organic matter contained in anoxic sediments by methanogenic archaeans. The quantity and the quality of organic matter, sediment texture and accumulation rates influence the type and abundance of the gas that is generated (Cavagna *et al*. 1999).

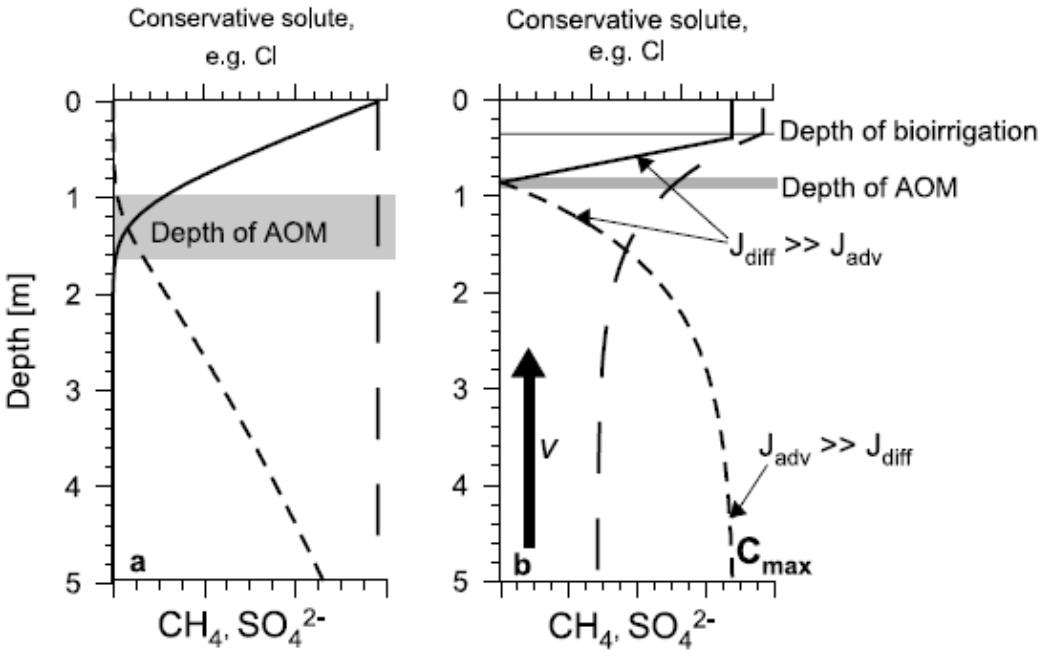

Figure 1.1.3. Schematic representations of the depth distribution of sulphate (-), methane (- -), and a conservative solute, e.g. chloride (——), in sediments not affected by fluid advection (a) and at seeps (b). In case of non-seep systems, solute transport is governed by molecular diffusion apart from potential bioirrigation. Fluid advection (v) at seeps leads to concave-down curvature of conservative solutes, if the advecting fluid is depleted in such solutes. Bioirrigation may be very intense at seeps, leading to a near homogeneous porewater surface layer. Coupled AOM and SR leads to concurrent consumption with steep gradients for sulphate and methane above the reaction zone respectively. A steep concentration gradient induces a high diffuse flux (J_{diff}) potentially exceeding the advecctive flux (J_{adv}). Note that methane is treated as a conservative solute below the depth of AOM. At depth, methane reaches asymptotically a maximum concentration C_{max}. From Ziebes & Haese (2005).

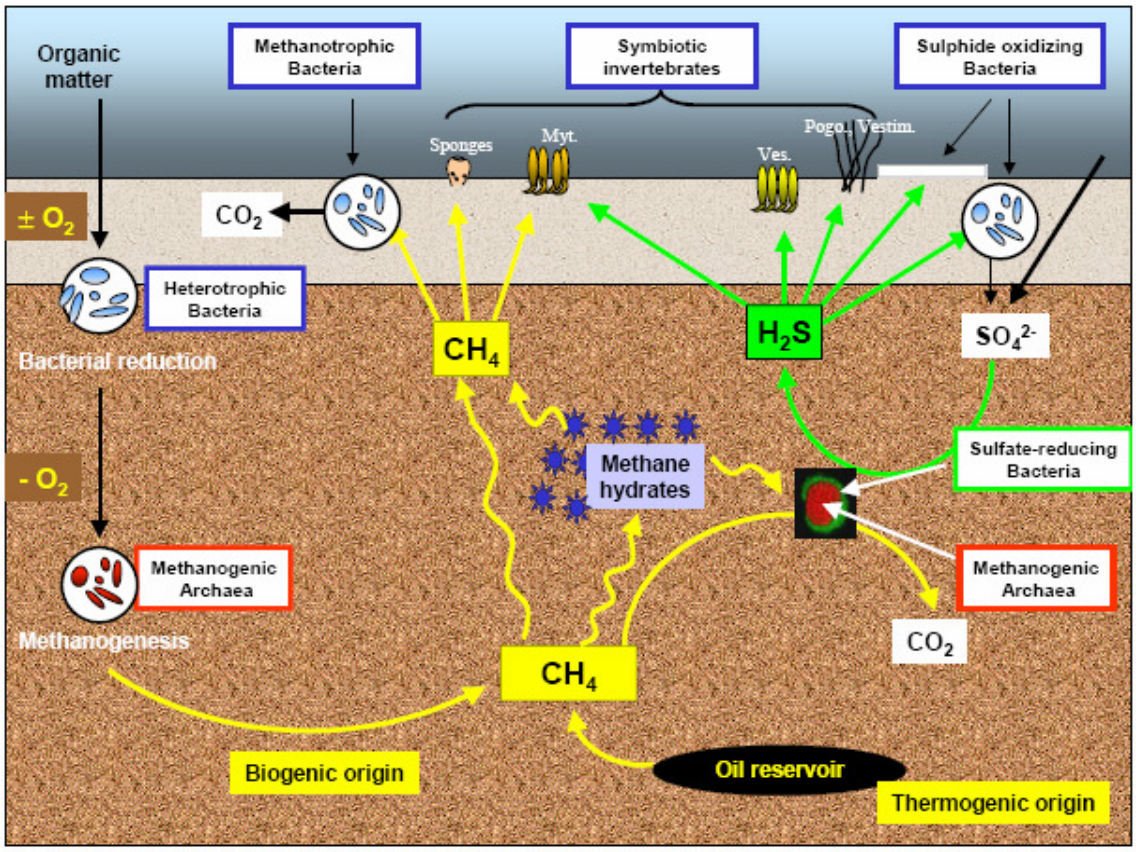

Figure 1.1.4. Role of methane at cold seeps. The methane (thermo- or biogenic) seeps through the sediment or is stocked as gas hydrates. The anaerobic oxidation of methane increases of hydrogen sulphide inside the sediment. Methane and hydrogen sulphide are the compounds used for the most part of symbiotic bacteria linked to metazoan that dominant the deep. From Duperron (2005).

Active sites in cold seeps are characterized by elevated concentrations of simple (C1-C5) and complex (oil) hydrocarbons, and in case of asphalt volcanoes associated with asphalt flows. The high concentration of sulphide in the sediments is mainly produced by microbial degradation of hydrocarbons other than methane coupled to the reduction of sulphate. A major type of cold seeps is represented by gas-emitting submarine mud volcanoes. Mud volcanoes are present at tectonically active and inactive areas of continental margins. These geological features result from the extrusion of fluid-rich mud flows (mud breccias) which, due to a deep pressure source, can form mounds and craters on the seafloor (Dimitrov 2002). The rising muds are often enriched in methane and with gas hydrates and represent a window to the deep biosphere and geosphere.

Because cold seeps are characterized by focused flows of the less readily metabolizable and poorly soluble gas methane and other hydrocarbons, they do not provide instantly usable carbon. Food sources, other than the rain of particulate organic carbon (POC) are complex and potentially toxic. Methane and hydrocarbons are turned into life nourishing elements by microbial communities that live i) free in the water; ii) in tissues of specialized benthic fauna; iii) in surface mats; and iv) in the sediment (Sassen 1993; Brooks *et al*. 1987; Aharon 2000).

These "hotspots" of increased biological activity on the seabed occur at a variety of spatial scales, from a few meters to several kilometers and are among the most heterogeneous of all continental margin ecosystems (Table 1.1.1). Sources of heterogeneity in cold seeps may arise from local variability in fluid flow, occurrence of gas hydrates, geochemistry, substratum and sediment stability. Carbonate precipitation, a by-product of microbial anaerobic hydrocarbon oxidation, and biogenic habitats created by foundation invertebrate species (symbiont bearing, sponges, soft and stony corals) provide substrata for a variety of macrofaunal and meiofaunal assemblages. These faunal assemblages respond to changes in structural complexity, habitat geochemistry, nutrient sources, and biotic interactions (Cordes *et al*. in press).

1.1.1.Biogeochemical processes in reducing habitats

In some reducing environments, especially vent systems, vast amounts of reduced substances as hydrogen, iron, magnese, and ammonium are emitted. The microbial oxidation of these compounds plays an important role in these ecosystems (Reysenbach & Shock 2002). These processes include methanogenesis, anaerobic and aerobic oxidations of methane, sulphate reduction and sulphide oxidation.

Table 1.1.1. Sources of habitat heterogeneity at reported cold seeps. Adapted from Cordes *et al*. (in press).

	Geological							Biological					
Region	Oil seeps	Mud volcanoes	Salt diapirs	Brine pools	Gas hydrates	Pockmarks	Carbonates	Vestimentiferans	Frenulates	Bathymodiolids	Vesicomyids	Other bivalves	Mats
East Pacific													
Aleutian Margin/Trench							X						
Hydrate Ridge					X		X				X		X
Eel River							X				X	X	X
Monterey Bay							X	X	X		X	X	X
Southern California	X	X			X		X				X	X	X
Guyamas Basin	X										X		X
Costa Rica Margin		X			X		X	X		X	X		X
Peru Margin/Trench											X		
Chile Margin					X		X	X			X	X	
West Pacific													
Sea of Okhotsk							X		X		X		X
Kurile Trench					X		X						
Japan Margin					X		X	X		X			
Japan Trench							X				X	X	
Edison Seamount							X	X		X			
Hikurangi Margin					X		X	X	X	X	X	X	X
Indian Ocean													
Makran, Pakistan		X			X		X		X		X		X
Sunda Arc					X		X		X		X	X	
East Atlantic													
Haakon Mosby		X			X				X		X		X
Storegga slide						X				X		X	X
Gulf of Cadiz		X	X		X		X		X	X	X	X	
Gulf of Guinea					X	X		X					
Angola Basin	X		X		X	X	X	X		X	X		X
Mediterranean Sea													
Calabrian Arc		X											
Mediterranean Ridge		X		X				X		X	X		X
Anaximander Mountains		X						X					
Nile Deep Sea Fan		X				X							
West Atlantic													
Laurentian Fan											X		X
Blake Ridge			X		X					X	X		X
Gulf of Mexico	X	X	X	X	X	X	X	X	X	X	X	X	X
Barbados		X					X	X		X			X
Brazil	X												

1.1.1.1.Methanogenesis

Most methane in the ocean is biogenic, i.e. it is produced by thermogenic transformation of organic matter or by microbial methanogenesis. These processes play an important role in the degradation of organic compounds and are the terminal step of the carbon flow in anaerobic habitats (Zinder 1993). Microbial methanogenesis is a process mediated by a specialized group of microorganisms, the methanogenic archaea (*Methanobacteriales*, *Methanoccoccales*, *Methanomicrobiales*, *Methanosarcinales*, *Methanopyrales*). They are strict anaerobes and occur only in anoxic habitats. In the oxic zone of marine sediments organic material can be fully degraded to CO_2. Contrastingly, in oxygen depleted areas the mineralization of organic matter is carried out by a sequence of interacting bacterial groups where methanogenic archaea represent the last stage of the sequence. Carbon dioxide is one of the main carbon sources for subsurface microbial methanogenesis, but they can also use acetate. The process of microbial methanogenesis occurs in several enzymatic steps (Lösekann 2006). An example of the microbial methanogenesis is the formation of deep granites, dolomites, oil-fields and sandstones (Kotelnikova 2002).

1.1.1.2. Anaerobic Oxidation of Methane

The major sink of methane is the anaerobic oxidation where methane and sulphate are concurrently available. Large amounts of sulphide are formed due to this process. Because microorganisms capable of anaerobic oxidation of methane (AOM) have not been isolated to date, the link between methane oxidation and sulphate reduction has led to the hypothesis that AOM could be carried out by a consortium of methanogenic archaea and sulphate reducing bacteria (SRB) (Hoehler *et al*. 1994; Harder 1997; Hansen *et al*. 1998). Recent molecular studies (reviewed by Heijs 2005) have shown the existence of Archaea/SRB consortium (Figure 1.1.5), but cultivation experiments have been unable to demonstrate the growth of such archaea on methane as the sole carbon source under low hydrogen concentrations (Zatsepina & Buffet 1997; Valentine *et al*. 2000; Girguis *et al*. 2003). The main problem with the notion of sulphate-dependent AOM is that the overall process only yields 22

kjmol^{-1}; an amount that is barely sufficient for cellular maintenance and seemingly insufficient for growth (Hoehler *et al*. 2001). These low energy yields led to the suggestion of a second possibility: the existence of an unknown, high-energy yielding reaction pathway that is linked with methane oxidation and sulphate reduction (Zatsepina & Buffet 1997; Orphan *et al*. 2001).

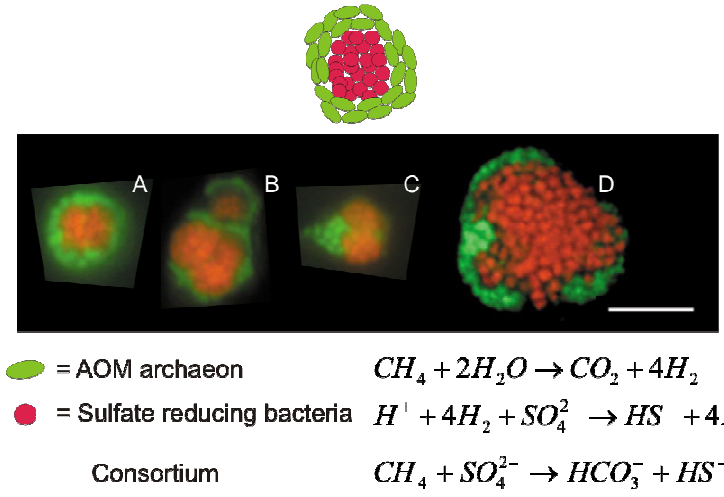

Figure 1.1.5. Schematic representation of the potential archaea/SRB consortium and their respective potential role in the anaerobic of methane. Photograph shows In situ identification of archaea/SRB aggregates with Fluorescently labeled rRNA-targeted oligonucleotide probes. The archaea are shown in red, and the SRB in green. After Heijs (2005) adapted from Boetius *et al*. (2000).

1.1.1.3.Aerobic Oxidation of Methane

The microbially mediated aerobic oxidation of methane (MOx) takes place in habitats where methane and oxygen are concurrently available. Microniches supporting the MOx are very restricted in marine sediments and the aerobic oxidation of methane is only a minor sink for methane in the ocean.

The initial oxidation of methane is catalyzed by a class of enzymes named methane monooxygenases (Figure 1.1.6). These utilize two reduction equivalents, which come from cytochrome c, to split the O-O bonds of dioxygen. One of the oxygen atoms is reduced to H_2O, and the other is incorporated into methane to form methanol. Dehydrogenases will oxidize methanol to formaldehyde, formate, and CO_2 and end products (Hanson & Hanson 1996).

1.1.1.4.Sulphate reduction

In marine sediments the anaerobic degradation process of organic matter is the dissimilatory sulphate reduction mediated by sulphate-reducing bacteria (Jorgensen 1982). The reduction of sulphate to sulphide is an eight-electron reduction and proceeds through a number of intermediate stages. Sulphate has first to be activated by means of Adenosine-5'-triphosphate (ATP). The enzyme ATP sulphurylase catalyzes the attachment of the sulphate ion to a phosphate of ATP leading the formation of adenosine phosphosulphate (APS). The sulphate in APS is subsequently reduced to sulphide by the enzyme APS reductase with the release of adenosine monophosphate (AMP). Once sulphite is formed, sulphide is produced by the enzyme sulphite reductase (Madigan & Martinko 2006).

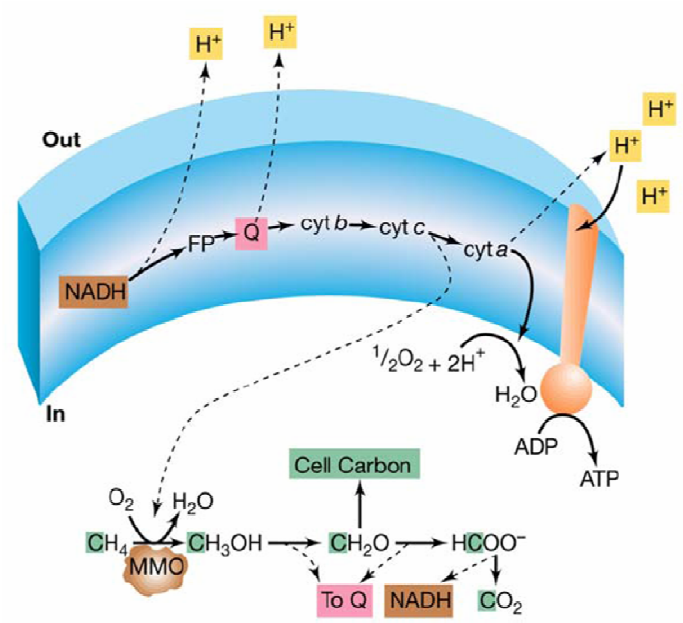

Figure 1.1.6. Oxidation of methane by aerobic methanotrophic bacteria. MMO: methane monooxygenase; FP: flavoprotein; Cyt: cytochrome; Q: quinine. Although not depicted as such, MMO is a membrane-associated enzyme. From Madigan & Martinko (2006).

Estimates of the overall significance of dissimilatory sulphate reduction for the degradation of organic matter suggest that the combined processes of aerobic respiration and sulphate reduction account for most of the organic carbon oxidation. The shape of the sulphate concentration profile through pore fluids provides a considerable amount of information about the carbon donor for bacterial sulphate reduction in the sediments (reviewed by Turchyn *et al*.

2006). When sulphate is consumed by organic matter oxidation (OMO), the sulphate concentration profile is typically concave down, reflecting the largely continuous consumption of sulphate with depth in the sediment. In sites where sulphate is consumed largely through AMO, sulphate is reduced at a single zone, which can be tens of meters below the sediment-water interface (Turchyn *et al*. 2006).

1.1.1.5. Sulphide oxidation

The oxidation of sulphide (SOx) is a process that requires the coexistence of sulphide and oxygen or nitrate (Fossing *et al*. 1995). Environments in which these conditions exist are the chemocline of stratified water bodies, marine or liminic sediments where the accumulation of organic matter has led to intensive sulphide production and hydrothermal vents where sulphide-rich, reduced fluids are emitted. Microorganisms can also use other common reduced sulphur compounds such as elemental sulphur or thiosulphate as electron donors but the final product of sulphur compound oxidation is in most cases sulphate. The oxidation of sulphide and sulphur involves first the reaction of these substances with sulfhydryl groups of the cell, such as glutathione with the formation of a sulphide-sulfhydryl complex (Figure 1.1.7).

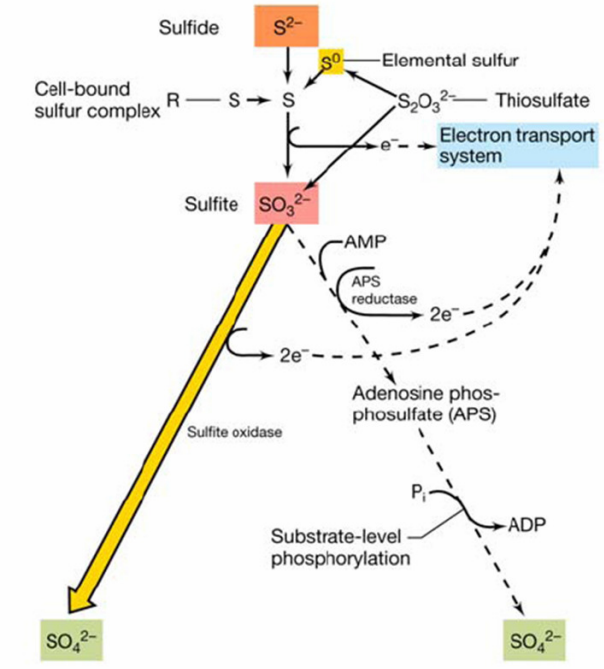

Figure 1.1.7. Oxidation of reduced sulphur compounds by sulphur-oxidizing bacteria. From Madigan & Martinko (2006)

The first oxidation step leads to the formation of elemental sulphur which is stored internally by many sulphur-oxidizing bacteria. The oxidation of elemental sulphur can bridge periods when sulphide is not available. The central intermediate during the oxidation of sulphide, elemental sulphur, and thiosulphate is sulphite and the carbon is fixed autotrophically via the Calvin Cycle (Madigan & Martinko 2006).

1.1.2.The inhabitants of reducing environments

The recent review of the ecology of cold seeps (Levin 2005) is a comprehensive account of the present knowledge on the structures faunal communities (abundance, composition, nutrition and behaviour of meiofauna and macrofauna) and how they interact with relevant geochemical and microbial processes.

There are only a few studies on meiofauna at cold seeps but these cover a variety of environments, water depths and geographic regions: North Fiji basin (Vanreusel *et al*. 1997); Hatsushima Central Japan (Shirayama & Ohta 1990); Monterey Bay cold seeps (Buck & Barry 1998); Gulf of Mexico (Robison *et al*. 2004); Barbados Prism (Olu *et al.* 1997), Haakon Mosby Mud Volcano (Van Gaever *et al*. 2006)

Major studies on seep macrofauna include the reviews by Sibuet & Olu (1998), Van Dover (2000), Kojima (2002) and Levin (2005) and other recent detailed studies carried out in different regions: Gulf of Mexico (Bergquist *et al*. 2003; Cordes *et al*. 2007); North Pacific Margin (Aleutian) Margin and the Gulf of Mexico (Levin & Mendoza 2007); Eastern Mediterranean Sea (Olu-Le Roy *et al*. 2004).

The dominant macrofaunal seep species are large symbiont-containing species that rely on sulphide or methane oxidation, or both, carried out by their chemoautotrophic bacterial symbionts (Sibuet & Olu 1998). Symbiont-containing macrofaunal species include Vesicomyidae, Mytilidae, Solemyidae, Thyasiridae and Lucinidae bivalves, Siboglinidae tube worms, and Cladorhizidae and Hymedesmiidae sponges. The occurrence of different taxa is

thought to be related to to the depth of methane/sulphide gradients in the sediment (Figure 1.1.8).

Most of the macrofauna studies for cold seeps focus extensively on these chemosynthetic species that account for the majority of the biomass in seeps, sometimes briefly mentioning some accompanying species. Taxonomic detailed studies are scarce making difficult the comparision among cold seeps communities.

The fauna living in and around cold seeps often display a spatial variability, depending on the distance to the seep (Bergquist *et al.* 2003). Biota inhabiting cold seeps include "obligate" species, restricted to sites in direct proximity to fluids rich in sulphide, methane, or other reducing inorganic compounds (e.g. ammonia; Fisher 1990), and "regional" species which occur in seeps and neighboring non-seep habitats. These sites are characterized by high microbial biomass (Guezennec & Fiala 1996) of obligate chemoautotrophic species (e.g. *Beggiatoa* sp.), and thiotrophic or methanotrophic symbiont-bearing invertebrates (Hovland & Judd 1988; Sibuet & Olu 1998). Other species such as galatheid crabs (*Munidopsis* spp.), limpets (*Pyropelta* spp.) and other gastropods (*Mitrella* spp.) may be heterotrophic but rely almost exclusively on chemosynthetic fauna (Barry *et al*. 1996). Regional fauna may forage on chemosynthetic biota (e.g. *Neptunea amianta*, lithodid crabs), but typically are not dependent on chemosynthetic production (Barry *et al*. 1996). Random guests (vagrants) are mostly predators that may exploit the elevated local production at seep sites but do not exhibit a consistent preference for them (Carney 1994).

Due to food availability for local fauna, higher biomass and abundance may occur at seeps compared to the low biomass and high diversity, found in the normal deep-sea (Agard *et al*. 1993). In her review of the ecology of cold seeps, Levin (2005) showed that total macrofaunal densities at seeps may be impoverished (North Sea: Dando *et al.* 1991), enhanced (Santa Barbara: David & Spies 1980; Oregon: Sahling *et al.* 2002; Gulf of Mexico: Levin *et al.* unpublished data), or identical (Levin *et al.* 2003) to those in nearby non-seep sediments.

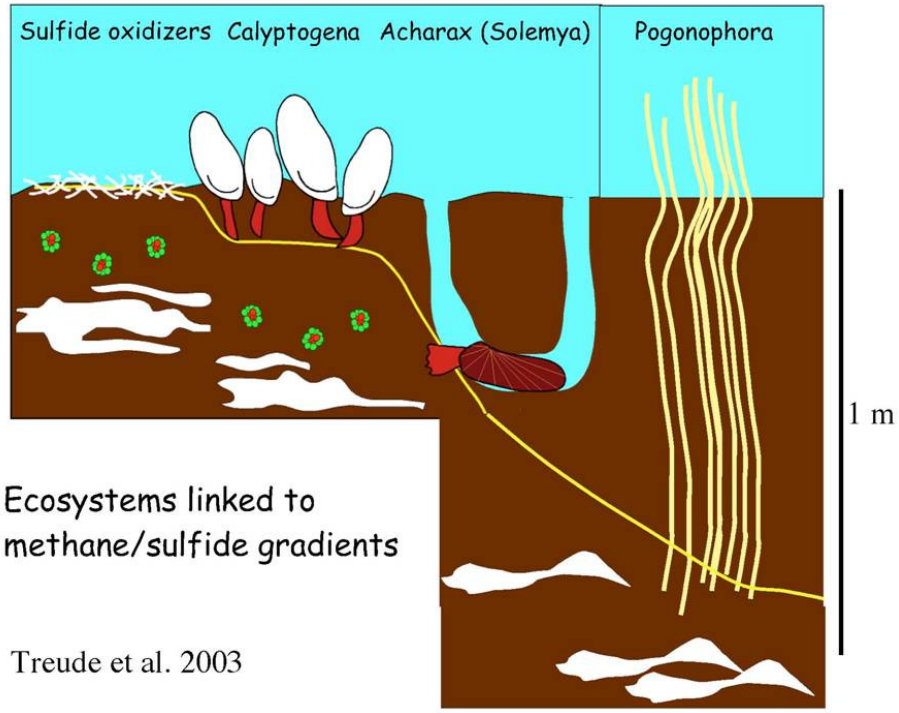

Figure 1.1.8. Scheme of the chemosynthetic communities at Hydrate Ridge. The yellow line represents the depth of the methane/sulphide gradient. From Treude *et al*. (2003)

1.1.3.Trophic relationships

Studies of the feeding relationships of deep-sea organisms are very limited and have depended primarily on stomach content analyses to determine the trophic status of species. Yet these data have substantial limitations. For example, gut contents reflect diets at particular points in time and space, and severely neglect certain types of dietary materials, such as gelatinous plankton and detritus that may nevertheless be very important in sustaining marine food-webs. Other limitations include problems associated with the voiding of gut contents upon capture, and the very sporadic feeding of many carnivore species. Furthermore, many animals crush or grind their food, such that identification of prey becomes very difficult, and it is not often clear which components of diets are actually assimilated, particularly when refractory or amorphous foods are ingested (Hobson & Welch 1992; Iken *et al*. 2001). Such difficulties are especially great in the study of food webs in the deep ocean (Fisher 1990; Polunin *et al*. 2001).

It has been demonstrated that the measurements of the abundances of naturally occurring stable isotopes of carbon ($^{13}C/^{12}C$) and particularly nitrogen ($^{15}N/^{14}N$) can provide trophic level information in marine food webs (Fry & Sherr 1988). This approach is based on the observation that, under certain circumstances, these isotopes undergo a predictable step-wise enrichment between prey and consumer tissues (Rau 1982; Wada *et al*. 1987; Fry 1988). The value of such tracers has been well established in many terrestrial and aquatic systems (reviewed by MacAvoy *et al*. 2002) including vent and seep environments (Conway *et al.* 1994; Van Dover & Fry 1994). It was the fact that vent tubeworms had very distinct $\delta^{13}C$ values relative to typical deep-ocean tissue carbon values that led Rau & Hedges (1979) to postulate that these dominant invertebrates had some non-photosynthetic food source.

Stable isotope approach offers distinct advantages over conventional dietary techniques since (1) trophic-level information is based on assimilates, not just ingested foods and (2) trophic positions represent long-term averages, and depending on the metabolic rate of the tissue that is being measured (Tieszen *et al.* 1983).

1.1.3.1.Food sources

Inputs of material to the deep ocean include falls from large animals such as sharks and marine mammals and export of coastal land-derived material (debris of plants and macroalgae), However the main input of primary production into this environment is considered to be what is loosely referred as "marine snow": the downward flux from the epilagic zone of photosynthetically fixed material, aggregated detrital material (e.g. faecal pellets and plankton exoskeletons) and zooplankton (reviewed by Polunin *et al.* 2001). The supply of food is generally considered as the main limiting factor for most deep-sea communities (Gage & Tyler 1991) and consequently marine snow constitutes a valuable food resource for deep-sea microbes, metazoans and bathyal detritivores.

The stable isotope ratios of vent and seep species have been crucial in suggesting that non-photosynthetically derived organic matter is being used and that element cycling within these communities may be complex (Figure 1.1.9). Several sources are available to vent and seep species from surface

and deep-water input, subsurface fluids, and pore water fluids. Potentially energy sources available to the infauna that inhabits bathyal reducing environments include photosynthetically-derived or carbon fixed chemosynthetically in the seabed, made available through the activities of free-living or symbiotic bacteria (Levin & Michener 2002). Sulphur oxidation (thiotrophy) and methane oxidation (methanotrophy) are the primary chemosynthetic pathways that support carbon fixation of significance to animals (Conway *et al.* 1994; Van Dover 2000). Thiotrophic bacteria oxidize hydrogen sulphide and utilize the energy released to fix carbon dioxide into organic compounds. Similarly, methanotrophs also use a reduced compound as an energy source, in this case methane, which they also use as carbon source (Fisher *et al*. 1987). The different carbon fixation pathways described above involve distinct isotopic fractionation which makes stable isotopic approaches particularly useful for elucidating nutritional modes of organisms in reducing deep-sea environments (Conway *et al.* 1994; Van Dover & Fry 1994).

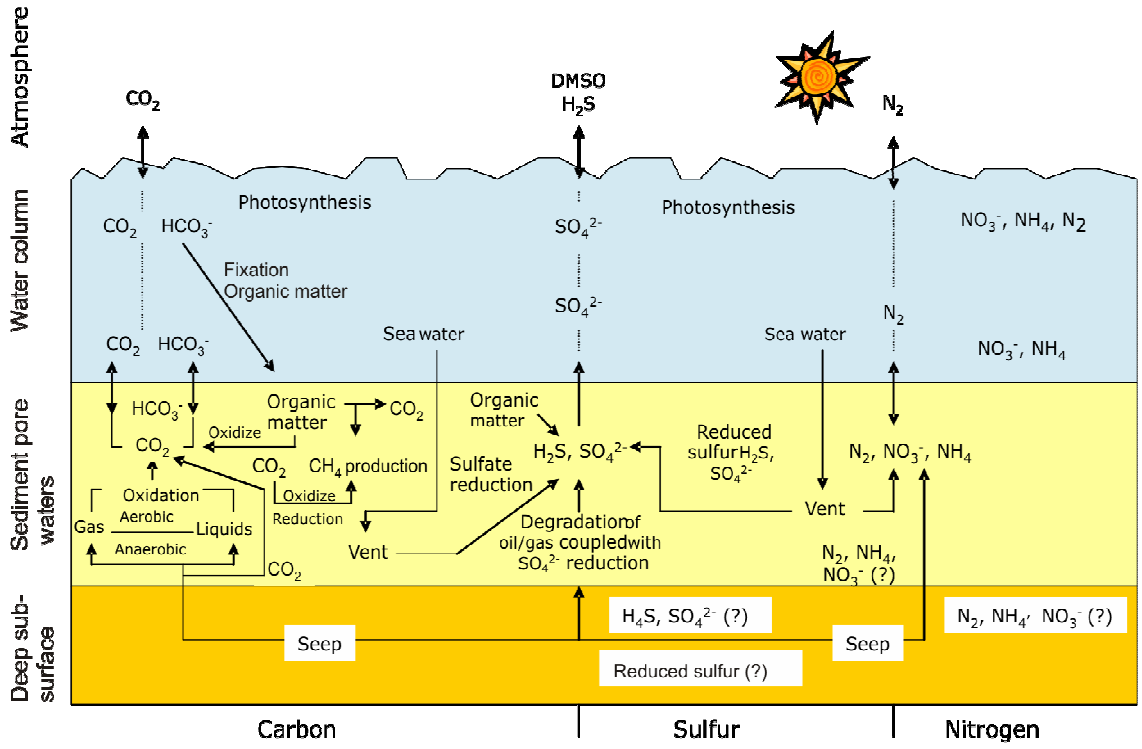

Figure 1.1.9. Element cycling in vent/seep communities. Adapted from Conway *et al.* (1994).

Nutrition of seep fauna varies, with thiotrophic and methanotrophic symbiotic bacteria fueling most of the megafaunal forms but macrofauna and most meiofauna are mainly heterotrophic. Macrofaunal food souces are largely photosynthesis-based at shallower seeps but reflect carbon fixation by chemosynthesis and considerable incoroporation of methane-derived carbon at deeper sites (Levin *et al*. 2000).

1.1.3.2.Stable Isotopes

Isotopes are atoms of a given element which nucleus contains the same proton numbers (Z) but a different neutron number (N). The atomic weight of each natural element is the weighted average of its isotopes. The isotopes might be divided in two fundamental types, the stable and the unstable (radioactive). The number of stable isotopes is around 300.

Isotopic composition is the relative distribution of isotopes of a given element, usually expressed in the form of the ratio of a less common isotope to the most common, such as $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$, and so on. Stable isotopes isotopic composition is expressed in terms of δ notation as per thousand (‰) differences from a standard reference material:

Equation 1 $$\delta X = \left[\frac{R_{Sample} - R_{s\tan dard}}{R_{s\tan dard}} \right] \times 10^3 \left({}^{0}\!/\!_{00} \right)$$

where δX is the isotope ratio in δ units relative to a standard, and R_{sample} and $R_{standard}$ are the absolute isotope ratios of the sample and standard, respectively. The use of a standard allows to equally reflecting any fluctuations in both standard and sample.

In ecology studies, the stable isotopes most frequently used are carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$), sulphur ($^{34}S/^{32}S$), oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$ or D/H). All isotope values reported in literature are referenced to primary standards. For carbon this is marine limestone fossil, Pee Dee Belemnite (or PDB) (Craig 1953). For nitrogen, the standard reference is atmospheric air (Mariotti 1983). The Vienna Standard Mean Ocean Water (V-SMOW) is the standard used for oxygen and hydrogen (Confiantini 1978),

while triolite from the Canyon Diabolo iron meteorite (CTD) is used as standard for sulphur (Krouse 1980).

Samples that contain more of the heavy isotope are referred to as "enriched" and are "heavier" than other samples. Those that contain less of the heavy isotope are "depleted" and are "lighter" than other samples. Consequently, positive values of δX indicate enrichment of the sample in the heavy isotope compared to the standard, and negative values imply depletion of the heavy isotope in the sample relative to the standard.

Isotopic fractionation involves the partial separation of isotopes during physical or chemical processes. This is produced by two main phenomena: (a) the kinetic isotopic effect, (b) the thermodynamic effect. The kinetic isotope effect is a variation in the reaction rate of a chemical reaction when an atom in one of the reactants is replaced by one of its isotopes.

1.1.3.3.The use of stable isotopes for food web analyses

Carbon isotopic compositions of animals reflect those of the diet within 1‰ (Peterson & Fry 1987). For this reason, stable-carbon isotope analysis is an ideal tool for tracing origins of nutrients in food webs since carbon may enter the base of the food web with characteristic isotopic signatures due to a variety of biogeochemical processes and these change little throughout the food web. The main processes of interest here are those resulting in differences in $\delta^{13}C$ values of chemosynthetic species and non chemosynthetic species.

The resource fuelling seep ecosystems is either methane or sulphide emanating from the sea floor. Tissue carbon isotopic values reflect the relative input of thermogenic and biogenic methane seepage at a given location. If methane-oxidizing symbionts incorporate methane carbon with little expression of isotopic fractionation, methanotrophic bacteria will have $\delta^{13}C$ ratios similar to the methane used. If the animal host is using endosymbiont carbon, host $\delta^{13}C$ ratios should also reflect the isotopes values of the source methane.

The carbon isotopic composition of biosynthetic organic matter is determined by the isotopic composition of the carbon source, the size of the CO_2 pool relative to the amount used (limitation) and the kinetic fractionations

associated with the assimilation of CO_2 during metabolism (Fisher 1990). The most significant degree of fractionation of carbon isotopes occurs during the initial steps of phototrophic reduction of inorganic carbon (i.e., carbon dioxide and bicarbonate) and results in a decreased $^{13}C/^{12}C$ isotope ratio in the organic product relative to that of the source carbon. Subsequent utilization of the organic carbon leads to small but detectable increases in the $^{13}C/^{12}C$ isotope ratio as the material passes through the food chain (Ruby *et al*. 1987).

Phytoplankton-derived organic matter typically has $\delta^{13}C$ signatures of -15‰ to -25‰ (Fry & Sherr 1984).Organisms with a diet based on carbon produced by marine chemoautotrophs will be expected to have $\delta^{13}C$ values considerably more negative than organisms whose diet is based on photossintetically fixed carbon, or less negative if in presence of Rubisco form II. In fact, carbon fixation fueled by energy derived from sulphide oxidation that involves Rubisco form I often produces $\delta^{13}C$ values between -27‰ and -37‰, but alternative pathways that involve Rubisco form II can yield much heavier $\delta^{13}C$ values (-9 to -16‰) (Brooks *et al.* 1987; Fisher 1990; Robinson & Cavanaugh 1995). Carbon isotopic values have been used to help differentiate animals with sulphur-oxidizing symbionts (–20 to –40‰) from those with methano-oxidizing symbionts (≤–40‰) (Brooks *et al.* 1987; Kennicutt *et al.* 1992) and to identify the source methane pool as either thermogenic ($\delta^{13}C$ = –40 to –45‰) or biogenic ($\delta^{13}C \leq$ –45‰) methane (Sassen *et al.* 1999).

Nitrogen isotopes can also be used to differentiate nutrient sources and provide an even more definitive indication of trophic level since there is a 3 to 3.5‰ enrichment in $\delta^{15}N$ per trophic level (Michener & Schell 1994). Animals in reduced settings that bear chemoautotrophic symbionts often exhibit light, sometimes negative $\delta^{15}N$ signatures if local inorganic nitrogen is assimilated or fixed (Conway *et al*. 1994). Chemoautotrophs tend to have lower $\delta^{15}N$ values (–5 to –12‰) than heterotrophs (2.8 to 13‰) or marine phytoplankton (MacAvoy *et al.* 2002). Unlike $\delta^{13}C$, which changes little as it is passed to consumers (~1 per mil trophic level-1) (Gearing 1991), nitrogen signatures increase by ~3.4‰ with each successive trophic level (DeNiro & Epstein

1978, 1981; Minagawa & Wada 1984). Therefore, both carbon and nitrogen isotopes combined can provide nutrient source and trophic information.

To support growth based on sulphide oxidation and carbon dioxide fixation, inorganic nitrogen (e.g. nitrate and ammonia) must also be assimilated. The mechanisms by which these symbioses assimilate ammonia and nitrate are not well characterized, but many of the transformations are probably localized to the bacterial symbionts. Many species of bacteria assimilate nitrate and ammonia into amino acids. In some species, nitrate serves both as an alternative electron acceptor and as a source of nitrogen. In both cases, nitrate is first reduced by nitrate reductase to nitrite which can subsequently be reduced to ammonia (reviewed by Lee *et al.* 1999).

Sulphur isotopes are also useful as nutrient tracers in systems where marine and freshwater nutrients mix (reviewed by MacAvoy *et al*. 2002). Additionally $\delta^{34}S$ is though to undergo minimal or no fractionation as a function of trophic level, making it an excellent tracer for nutrients since no correction due to trophic fractionation is needed. Some $\delta^{34}S$ measurements have also been made on seep chemoautotrophs and heterotrophs (Brooks *et al.* 1987; MacAvoy *et al.* 2002). The $\delta^{34}S$ values of the thiotrophic animals were depleted relative to the methanotrophic bivalves (13‰) and seep heterotrophs (13 to 16‰) (Brooks *et al.* 1987).

The extent of fractionation of stable carbon isotopes, as well as of stable sulphur isotopes, is affected by rates of metabolism, by temperature, and possibly by other growth parameters (reviewed by Ruby *et al*. 1987). Because of the great carbon fractionation associated with methanogenesis, a significant input of microbial methane into the food chain would have a major effect on depressing the $^{13}C/^{12}C$ ratio in the associated heterotrophic biomass (Ruby *et al*. 1987).

During the past decades stable isotope techniques have been currently used to infer trophic relationships at hydrothermal vents (Van Dover & Fry 1989, 1994; Vetter & Fry 1998; Van Dover 2002; Colaço *et al*. 2002; Yamanaka *et al*. 2003; Levesque *et al.* 2006), at whale falls (Deming *et al*. 1997) and at cold seeps in California (Levin & Michener 2002; Levin *et al*. 2003), Gulf of Mexico (MacAvoy *et al*. 2002, 2003, 2005; Levin & Mendoza

2007), Haakon Mosby Mud volcano (Gebruk *et al*. 2003) and Eastern Mediterranean (Werne *et al*. 2002; Olu-Le Roy *et al*. 2004)

It is generally believed that chemosynthetic primary production forms the base of the hydrocarbon seep community food web; the study of chemosymbioses is of great importance to understand the foundation and role of this kind of nutrition in these systems.

1.1.4.Chemosymbioses

Within the deep-sea, highly productive communities associated with patchy, energy-rich hydrothermal vents and cold seeps have drawn considerable interest due to their dependence upon microbial chemosynthesis. The term was coined by Pfeffer (1897) in obvious contrast to the already well-known photosynthesis. Both processes involve the byosynthesis of organic carbon compounds from $C0_2$, with the source of energy being either chemical oxidations or light, respectively (Jannasch & Mottl 1985). In this process prokaryotic organisms synthesize C3 compounds (e.g. phosphoglycerate) from C1 compounds (e.g. CO_2, CH_4) using chemical energy. During chemosynthesis, the energy required for basal metabolism and growth is derived from the enzyme-catalyzed oxidation of reduced chemical species (e.g, sulphide, elemental suphur, thiosulphate, methane, hydrogen and ammonium) (Kennicut *et al*. 1992). Carbon fixation is accomplished through the Calvin cycle, catalyzed by the enzyme ribulose 1,5 bisphosphate carboxylase (RuBisCO), similar to the one used by photosynthetic organisms. In chemosynthesis two forms of Rubisco are common: form I adapted to oxic conditions and form II more adapted to anoxic conditions (Robinson & Cavanaugh 1995).

The seemingly inhospitable hydrothermal vents and hydrocarbon seeps have been found to be inhabited by a remarkably abundant benthic fauna, which have adopted chemosymbiosis as the principal mode of nutrition (Jannash & Mottl 1985; Kennicutt *et al*. 1985; Tunnicliffe 1992).

The morphology of the symbioses varies widely as might be expected from the diverse phylogenetic origins of the hosts. However, a common

characteristic is that in host species the digestive system is absent or reduced in function. Thus given the inability of the host to feed on particulate organic matter, the bacterial population is the primary mode of carbon acquisition. In spite of some success in enrichment essays, until today, pure cultures of symbionts are not available (Campbell *et al*. 2001). Much of what is known about the symbionts is based on the study of intact symbioses by transmission electron microscopy, radiotracer experiments, stable carbon isotope signatures, and enzyme activity assays (Cavanaugh *et al*. 2006).

Sulphur-based symbioses are by far the most common chemosynthetic symbioses known to date (Nelson & Fisher 1995). In thiotrophic animals, energy utilized by the symbiont is derived from the oxidation of reduced sulphur (H_2S, thiosulphate, etc.) and carbon is derived from dissolved inorganic carbon (DIC) from seawater, ventwater or sediment pore water (Kennicut *et al*. 1992). Methane-based symbioses are only found in very few metazoan species, although the oxidation of methane and sulphide yield similar free energies. Constraints on methane-based symbioses are mostly linked to the concentration of methane in the environment or its biological availability. Most host species have evolved abilities to bind sulphide and concentrate it above ambient levels, whereas such capabilities are not known for methane (Lösekann 2006).

1.1.4.1. Symbiotic-bearing invertebrates

A symbiotic association between sulphur-oxidizing chemoautotrophic bacteria and marine invertebrates was first proposed for the deep-sea hydrothermal vent tubeworm, *Riftia pachyptila* (Cavanaugh *et al*. 1981; Felbeck 1981). But the knowledge of the symbiotic associations have greatly increased during the past decade and several symbiotic associations with chemoautotrophic, sulphur-oxidizing and methanotrophic bacteria are now known to occur in a wide taxonomic range of invertebrates (Table 1.1.2). In the reducing environments at redox boundaries were reduced compounds and oxygen are present in close proximity, symbiotic bacteria have a better access to reduced compounds such as methane or sulphide and to oxidants enabling them to fix CO_2, while the eukariotic hosts acquire carbon from the symbionts either through translocation of nutrients or direct digestion of bacteria.

Symbioses between bacteria and eukaryotes impact the physiology, ecology and evolution of metazoan organisms (Krueger & Cavanaugh 1997; Stewart *et al*. 2005).

A brief account on several symbiotic associations for different taxa is given below; since the majority of studies were carried out for hydrothermal vent species, this summary includes mostly references to hydrothermal vent species. Symbiotic associations in siboglinid tubeworms and bivalves from seeps will be described in more detail.

Porifera

All sponge species examined to date are associated with microsymbionts, and in the case of 'bacteriosponges' the symbionts may constitute more than one-third of the total tissue volume (Vacelet 1975; Reiswig 1981). Symbiosis between methane-oxidizing bacteria and a deep-sea carnivorous cladorhizid sponge have been reported from mud volcanoes in the Barbados Trench. The sponge tissue contains 2 main morphological types of extracellular symbiotic bacteria (Vacelet *et al*. 1995). In another species a partnership with Crenarchaeota was described and was the first evidence of symbioses with the marine archaea (Preston *et al*. 1996).

Annelida (except siboglinids)

Inandrilus and *Olavius* are two gutless oligochaeta. *Inanidrilus leukodermatus* is one of the best studied gutless oligochaetes and is commonly found in intertidal and subtidal calcareous sands in the coral reefs around Bermuda and along the coast of Belize (Dubilier *et al*. 1995) and in cold seeps (Blazejak *et al*. 2005). The bacterial symbionts occur just below the outer cuticle of the worm inbetween extensions of the epidermal cells and are, in most cases, extracellular (Giere *et al*. 1981; Dubilier *et al*. 1995; Giere *et al*. 1995). *Delta*, *Alpha* and *Gamma-proteobacteria* and spirochetes that were found to occur in these species, participate in the sulphur cycle, and the coexistence of spatially separated sulphide-oxidizing and sulphate-reducing symbionts can explain why these animals survive on the oxic-anoxic interface (Blajezak *et al*. 2005).

Alvinella pompejana is a polychaetous annelid that inhabits narrow tubes along the walls of high-temperature hydrothermal vent chimneys of the East

Pacific Rise (Desbruyères *et al*. 1998). The worm hosts a rich community of epibiotic bacteria that coats its dorsal surface (Haddad *et al*. 1995), but its biomass is dominated by filamentous, *Epsilon-Proteobacteria* (Haddad *et al*. 1995; Campbell & Cary 2001). The epibiont community has been hypothesized to detoxify the worm's presumably poisonous microenvironment (Alayse-Danet *et al.* 1987), provide a nutrient source for its host (Alayse-Danet *et al.* 1986), or thermally insulate the host from its high-temperature environment (Desbruyères *et al*. 1998). Nevertheless Di Meo-Savoie *et al*. (2004) showed that sulphur is mobilized as the less toxic form of iron sulphur. Sulphur-oxidizing bacteria (Cottrell & Cary 1999) linked to nitrogen cycle (Jeanthon & Prieur 1990) and spirochetes that take effect on available hydrocarbons (Campbell & Cary 2001) form the rest of the bacterial community.

Mollusca (except bivalves)

The gastropods *Ifremeria nautilei* and *Alvinoconcha hessleri* live in high abundance around deep-sea hydrothermal vents of the Western Pacific. Ctenidia of *Ifremeria nautilei* specimens harbor sulphur-oxidizing bacteria (Windoffer & Giere 1997). Some specimens from Manus basin also possess active methanotrophs (Galchenko *et al*. 1992). Symbionts of *Alvinoconcha hessleri* belong to *Epsilon-proteobacteria*, probably linked to sulphur cycle (Urakawa *et al*. 2005), and some use the cycle "TCA reverse" to carbon fixation (Suzuki *et al*. 2005). Symbionts are the nutrition basis for *A. hesseleri*; whereas *I. nautili* seems to have a mixotroph regime (Pranal *et al*. 1996).

Two species of Solenogastres (Mollusca, Aplacophora) from deep-sea hydrothermal vents of the East Pacific Rise were discovered to harbor epibiotic and endocuticular prokaryotes along their entire bodies. The majority of the prokaryotes associated were identified as *Alpha-Proteobacteria*, while others belonged to the *Gamma-Proteobacteria* or could not be associated with a definite bacterial clade (Katz *et al*. 2006).

Crustacea

Rimicaris exoculata is an exclusive species from hydrothermal vents from Mid-Atlantic Ridge. First feeding evidence suggested that these animals nourish on microbial mats presents on mineral surfaces of the chimneys (Van

Dover *et al*. 1988) but the presence of a gill chamber and a reduced gut showed evidence for a symbiotic nutrition (Segonzac *et al*. 1993). In fact the branchial gills harbour a monoculture of epibiotic filamentous sulphur-oxidizing bacteria (Polz & Cavanaugh 1995). Furthermore the observation of deposits of metallic oxides linked to water flow on the gill chamber suggests the probable presence of iron bacteria (Zbinden *et al*. 2004). The digestive tube harbours at least seven bacteria phylotypes, mainly *Epsilon-proteobacteria*, entoplasmatales (linked to insect symbionts), deferribacteres (which metabolizes metals, therefore the true iron reducers), Spiroplasma and Geovibrio (Zbinden & Cambon-Bonavita 2003).

Echinodermata

Many species of echinoderms, in all five extant classes, contain subcuticular bacterial symbionts (SCB). While there is some suggestion that SCB may be involved with nitrogen metabolism, understanding of the basis of the symbiosis, as well as the possible advantages or disadvantages to either hosts or symbionts, is limited (Lesser & Blakemore 1990; Burnett & McKenzie 1997). While SCB do not appear to be closely related to any previously described group of symbionts of marine invertebrates, they are closely related to intracellular plant symbionts and pathogens in a tightly clustered subgroup of the a subdivision of the Proteobacteria designated Alpha-2 (Woese 1987). This group includes the rhizobacteria (*Rhizobium* spp.) and the agrobacteria (*Agrobacterium* spp.). The rhizobacteria include many nitrogen-fixing plant symbionts, while the agrobacteria are important plant pathogens. The possible role of SCB in nitrogen metabolism and their ecological niche as endosymbionts are therefore consistent with their hypothesized phylogenetic position. It is interesting, however, that SCB appear to be the only examples of extracellular symbionts among a subdivision of the Proteobacteria thus far described (Burnett & McKenzie 1997).

Table 1.1.2. List of taxa hosting chemosynthetic bacterial symbionts. Habitat: Cold Seeps (CS), Hydrothermal vents (HV), Whale falls (WF), Other Reducing environments (RE); Metabolism: sulphur-oxidizing (SOX), methano-oxidizing (MOX), iron-oxidizing (FeOX); Localization: extracellular (E), intracellular (I); Transmission: environmental (Env), Vertical (Ver). Data from Duperron (2005) Stewart *et al.* (2005).

Taxa		Host Occurrence	Habitat	Digestive tube	Symbiont Types	Metabolism	Location	Position	Transmission
Protozoa	Ciliata		CS, mangrove swamp		?	SOX	Host cell surface	Epibiotic	?
Porifera	Cladorhizidae	*Cladorhiza* sp.	CS		?	MOX	Skeletal matrix	E	?
Nematoda	Stilbonematinae	Nematoda			*Gamma*	SOX	Cuticle	Epibiotic	?
	Solemyidae	All species	RE, HV, CS	Reduced	*Gamma*	SOX	Gills		Ver
	Lucinidae	All species	RE, CS	Reduced	*Gamma*	SOX	Gills	I	Env
Bivalvia	Thyasiridae	Some species	RE, CS	Reduced	*Gamma*	SOX	Gills	I, E	Env
	Vesycomidae	Some species	HV, CS	Reduced	*Gamma*	SOX	Gills	I	Ver
	Mytilidae	*Bathymodiolus*	HV, CS	Reduced	*Gamma*	SOX, MOX	Gills	I, E	Env (SOX)
Gastropoda	Provannidae	*Alviniconcha Ifremeria*	HV (Pacific)	More or less reduced	*Gamma, Epsilon*	SOX (MOX?)	Gills	I	Env
	Lepetodrilidae		HV			SOX	Gills	Epibiotic	
Annellida	Alvinellidae	*Alvinella*	HV (E Pacific)	Present	*Epsilon*	SOX	Cuticle	E	?
	Siboglinidae	Vestimentifera, Frenulata, Monilifera *Osedax*	HV, CS, WF	Specialized (trophossome)	*Gamma*	SOX, MOX	Trophosome, zone subannular	I	Env?
	Oligochaeta	*Inanidrilus, Olavius*	Coralline sands, CS	Absent	*Gamma, Alpha, Delta*	SOX, SRB	Subcuticular	E	
Arthropoda	Crustacea	*Rimicaris*	HV (Atlantic)	Present	*Epsilon, Defferibacteri*a	SOX (FeOX)	Gill chamber	E	?
Echinodermata	Several	Several	RE	Present	*Epsilon*	SOX	Gut	E	Env

1.1.4.2.Symbiotic-bearing siboglinids

All members of the family Siboglinidae live in obligate symbiosis with bacterial endosymbionts and rely on the chemosynthetic capabilities of their symbionts for nutrition (Felbeck 1981; Southward *et al*. 1986). Most siboglinids are thought to host a single species of chemosynthetic endosymbiont, which they obligatory depend upon for organic nutrition (Distel *et al*. 1988; Vrijenhoek *et al*. 2007). The symbionts are harbored in an interior vascularized tissue called the trophossome (Southward 1982). The tubeworms provide their symbionts with both oxygen from the water column and energy-rich compounds from the anoxic sediments.

Siboglinid endosymbionts are typically thought to fall into one of three general metabolic categories: (1) thiotrophic symbionts that oxidize sulphide or other reduced inorganic compounds and fix CO_2 by the Calvin-Benson cycle, (2) methanotrophic symbionts that use methane as both the source of energy for metabolism and the carbon for assimilation, or (3) heterotrophic symbionts found in the bone-eating worms, *Osedax* spp. (Fisher 1990; Stewart *et al*. 2005; Goffredi *et al*. 2007).

To date, the symbiotic associations of vestimentiferans of hydrothermal vents are well studied with molecular methods and sequences of many host species and their symbionts are published (Naganuma *et al*. 1997; Di Meo *et al.* 2000; Kimura *et al.* 2003; McMullin *et al*. 2003).

Lamellibrachia is a common seep vestimentiferan, which obtains sulphide from the sediment using long, root-like, posterior extensions of its body (Julian *et al*. 1999; Freytag *et al*. 2001). These seep vestimentiferans grow slower than their hydrothermal relatives and may reach 100 to 200 years of longevity (Fisher *et al*. 1997). Large aggregations can have extensive networks of roots more than a meter deep in the underlying sediment (Figure 1.1.10). Microbial sulphate reduction depletes sulphate and hydrogen ions from the sediment pore-water surrounding *Lamellibrachia* roots, creating a favorable gradient to these ions to diffuse out of the roots. These animals can avoid the high energetic demands of sulphate and proton elimination across its plume by passive transport of these ions across its roots (Dattagupta *et al.*

2006). A large diversity of bacteria (*Alpha*, *Gamma*, *Epsilon proteobacteria*) is associated to *Lamellibrachia* (Kimura *et al*. 2003).

Frenulata worms account for 75% of all recognized siboglinids. In comparison to vestimentiferans, frenulates are typically minute, about the thickness of a coarse human hair, and more than 10–20cm long (Southward *et al*. 2005). They are found throughout the world's oceans, along continental margins, continental slopes, fjords, trenches, mud volcanoes, and in the periphery of other cold seeps and vents (reviewed by Thornhill *et al*. 2008) and their symbiotic associations are poorly known.

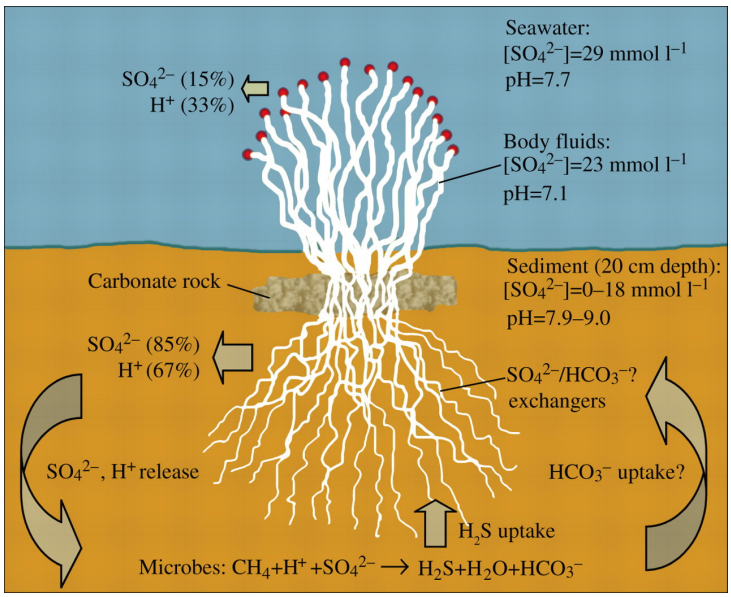

Figure 1.1.10. A schematic representation of an aggregation of *Lamellibrachia luymesi*, depicting a nutrient exchange model for this species. Seawater and sediment pore-water sulphate concentration and pH are from Aharon & Fu (2000). The equation for microbial sulphate reduction shows sulphate reduction coupled with methane oxidation. However, sulphate reduction coupled with higher molecular mass hydrocarbons can also occur (Joye *et al*. 2004). Bicarbonate uptake across roots is suggested, but has not been directly demonstrated in this study. From Dattagupta *et al*. (2006)

Studies on the symbioses of Frenulata and Monilifera are still scarce. In contrast to previous studies indicating that *Sclerolinum contortum* is associated with methane-oxidizing symbiont (Pimenov *et al*. 1999), Lösekann *et al*. (2008) only detected sulphur-oxidizing symbionts in this species. On the same study symbionts of *Oligobrachia haakonmosbiensis* were also determined as belonging to the *Gammaproteobacteria*. On both species the

microbial diversity was very low; the low number of symbiotic cells might me attributed to a slow-growing lifestyle of these animals with a correspondingly low energy demand (Lösekann *et al*. 2008). For *Oligobrachia mashikoi* the phylogenetic analysis indicates that the seven phylotypes are related to the environmental clones, closely connected to *Gammaproteobacteria* (Kubota *et al*. 2007). Symbionts of *Siboglinum fiordium* from Norway were closely related to symbionts of *O. mashikoi* from Japan and is suggested that symbionts of frenulates may share common evolutionary history or metabolic features (Thornhill *et al*. 2008).

1.1.4.3.Symbiotic-bearing bivalves

The dominant bivalves at cold seeps are large chemosymbiotic species belonging to the families Vesicomyidae and Mytilidae with Thyasiridae, Solemyidae and Lucinidae sometimes also abundant.

Most clams and mussels (e.g. *Calyptogena* spp., *Acharax* sp.) harbor chemoautotrophic, sulphur-oxidizing symbionts inside specialized gill cells (bacteriocytes) where they are in close contact with the surrounding seawater. These animals typically concentrate sulphide through their foot which is in contact with reduced fluids. Toxic effects of sulphide are prevented by the presence of sulphide-bonding transport proteins other than hemoglobin or by the activity of sulphide oxidases which transform sulphide to the less toxic form of thiosulphate (Felbeck & Distel 1992).

Solemyidae

The family Solemyidae is a group of ancestral protobranch bivalves that are found throughout the world's oceans, in shallow water and deep-sea habitats (mainly cold seeps), and in temperate and tropical regions (Vokes 1955; Parker 1982; Tunnicliffe *et al*. 2003). These bivalves live burrowed in Y or U shape tubes, and their digestive tract and labial palps are reduced, with some species lacking completely an alimentary canal (Bernard 1980). The taxonomy of the Solemyidae is difficult; the genus *Acharax* differs morphologically from *Solemya* in having an external ligament set on a narrownymph (Coan *et al*. 2000). However, at a first glance both solemyids appear so similar that specimens discovered at various deep-sea sites might have been misclassified

erroneously as *Solemya*. Recently some of the former *Solemya* species were included in the genus *Petrasma*. (Taylor *et al*. 2009). As far as it is known, all species of the Solemyidae live in symbiosis with chemoautotrophic sulphur-oxidizing bacteria, on which they depend to varying degrees (Anderson *et al*. 1987; Gustafson & Reid 1988; Fisher 1990). The thioautotrophic metabolism of solemyid symbioses depends on the availability of sulphide in the silty sediments in which these associations occur.

The genus *Acharax* is widespread and it has been found at several cold seeps (Sibuet & Olu 1998; Neulinger *et al.* 2006) and in sediments influenced by hydrothermal venting (Juniper *et al*. 1992; Métivier & von Cosel 1993). The genus *Solemya* lives in sediments with high organic matter content, often at reduced oxygen concentrations. Hydrogen sulphide is frequently present due to sulphate reduction coupled with organic matter degradation (Conway *et al*. 1992). After the discovery of the symbiotic chemoautotrophic bacteria, numerous studies of *Solemya* enlightened the physiology of the symbiosis as well as the phylogeny and transmission of the endosymbionts (reviewed by Neulinger *et al*. 2006).

Enzymmatic, physiological, and molecular data for several species of *Solemya* suggest that fixation of carbon dioxide occurs primarily via ribulose 1,5-bisphosphate carboxylase–oxygenase (RubisCO) in the Calvin Benson cycle, using ATP and NADPH generated from sulphur oxidation. Anapleurotic pathways in both host and symbiont may fix lesser amounts of CO_2. Transfer of organic matter from symbionts to host likely occurs via translocation of simple nutritive compounds (e.g., amino acids) released by the bacteria (Fisher & Childress 1986), though direct digestion of symbiont cells may also take place (Stewart & Cavanaugh 2006).

In most solemyid symbioses studied to date, the symbionts are concentrated apically within the bacteriocytes, presumably to facilitate access to metabolic substrates derived from the external environment. However, symbiont distribution may vary depending on the size of the host organism.

Maternal transmission in solemyid symbioses undoubtedly strongly impacts the evolution of the bacterial symbiont. Such diversity among chemoautotrophic symbionts is unprecedented within a single bivalve genus

and argues against a hypothesis of host-symbiont cospeciation for *Solemya*, in contrast to the Vesicomyidae and Lucinidae (Krueger & Cavanaugh 1997).

The phylogenetic relationships indicate that *Solemya* and *Acharax* have the most ancient ancestor of symbiotic sulphur bacteria and that vestimentiferan and lucinidan symbionts evolved later, possibly from symbionts harboured by ancestors of present-day *Solemya* spp (Imhoff *et al*. 2003).

Lucinidae

The Lucinidae, in which the sulphide-oxidizing symbiosis is likely obligate, is by far the most disparate and species-rich family occupying a great variety of habitats over a broad geographical range (60°N−55°S). Some species are associated with cold seeps and mud volcanoes (Carney 1994; Callender & Powell 1997; Salas & Woodside 2002), with oxygen minimum zones (Cary *et al*. 1989; Oliver & Holmes 2006) and a single species (*Bathyaustriella thionipta*) with a hydrothermal vent (Glover *et al*. 2004). Lucinids display typical characteristics of symbiont-associated bivalves, such as reduced gut and palps and large fleshy gills (Fiala-Médioni & Felbeck 1990; Taylor & Glover 2000).

Phylogenetic characterization of the symbionts has shown that they belong to the *Gammaproteobacteria*, and are related to symbionts from thyasirid and solemyid bivalves as well as symbionts from siboglinid tubeworms (Stewart *et al*. 2005). A spirochete phylotype not close related spirochete symbionts previously identified in the oligochaetes *Olavius algarvensis* and *Olavius ilvae* has also been identified in *Lucinoma kazani* (Duperron *et al*. 2007). Contrarily to most of the other symbiotic bacteria, which use oxygen as electron acceptor for respiration, symbionts from *Lucinoma aequizonata* utilize nitrate (Hentschel & Felbeck 1995). This anaerobic respiration is an isolate adaptation to the environment; other lucinids, such as *Codakia orbicularis,* consume the abundant oxygen present in its habitat (Duplessis *et al*. 2004).

Thyasiridae

As opposed to many other bivalves with chemoautotrophic symbionts, thyasirids are small (most species are small at less than 10 mm) (Dufour 2005). Thyasirids show a varying nutritional dependence on symbiosis (Dando & Spiro 1993; Dufour & Felbeck 2006) and not all thyasirids possess a

symbiosis with sulphide-oxidizing bacteria. In fact 15 of the 26 species examined by Dufour (2005) were asymbiotic.

For those thyasirids possessing symbionts the bacteria are housed extracellularly, either en masse in large apical vacuoles of bacteriocytes or amongst the microvilli (reviewed by Taylor *et al*. 2007). An exception is *Maorithyas hadalis* from hadal depths off Japan where the bacteria are housed intracellularly in membrane-bound vacuoles (Fujiwara *et al*. 2001). However, molecular confirmation is needed that this species is correctly placed in the Thyasiridae (Taylor *et al*. 2007).

Vesicomyidae

Bivalves in the family Vesicomyidae are common members of chemosynthetic communities and seep habitats worldwide (Lutz & Kennish 1993; Sibuet & Olu 1998). All species known (>60) are nutritionally dependent upon sulphide-oxidizing bacterial endosymbionts housed in gill tissues. Symbionts of Vesicomyidae display much smaller phylogenetic distances than other clam symbionts, form a coherent phylogenetic group, and were proposed to be of monophyletic origin (Distel *et al*. 1994; Peek *et al*. 1998).

Most vesicomyids have been identified from seep environments, where they are a common dominant megafaunal taxon, particularly along the eastern and western Pacific margin (Sibuet & Olu 1998; Sahling *et al*. 2002; Barry *et al*. 2007), but also in the Atlantic (Olu-Le Roy *et al.* 2007). The genus *Calyptogena* is one of the most studied. The bacteria-containing gill of *Calyptogena* with its entirely reduced outer demibranchs and wide interlamellar septa is one of the most specialized in the family Vesicomyidae (Krylova & Sahling 2006). The genera *Vesicomya* and *Isorropodon* are also frequent at cold seeps (Corselli & Basso 1996; Cosel & Salas 2001; Olu-Le Roy *et al*. 2004) but, up to now, there are no studies on chemosymbioses for these species.

Mytilidae

Bathymodioline mussels occur in chemosynthesis-based ecosystems such as cold seeps, hydrothermal vents and other reducing environments worldwide. Two phylogenetically and physiologically distinct types of

Gammaproteobacteria occur as symbionts in bathymodioline mussels: small chemoautotrophic bacteria shown to oxidize sulphide in at least one species (Nelson *et al*. 1995), and methanotrophic bacteria with typical stacked internal membranes that use methane both as an electron and a carbon source (Cavanaugh *et al*. 1987; Fisher 1993; Distel & Cavanaugh 1994). Some bathymodioline mussels harbour only a thiotrophic or a methanotrophic symbiont, while others live in a dual symbiosis, with both types of bacteria co-occurring in the gill bacteriocytes (DeChaine & Cavanaugh 2005; Duperron *et al*. 2006)

Six distinct bacterial 16S rRNA phylotypes, including four bacterial symbionts in the large seep species *Bathymodiolus heckerae* (Duperron *et al*. 2007) and another two belonging to groups not yet reported as symbionts in mytilids, in the small *Idas* from the Eastern Mediterranean (Duperron *et al*. 2008) were recently discovered. This discovery suggests that symbiont diversity in mytilids has probably been underestimated, and questions whether or not the common ancestor of bathymodioline mussels was associated with multiple bacteria (Duperron *et al*. 2008).

1.1.4.4.Bacterial key players

The development of molecular biology techniques allowed several studies which increase the knowledge of several symbiosis aspects such as diversity, functioning, ecology and evolution (Imhoff *et al*. 2003).

Based on 16S rRNA phylogeny, most symbionts belong to the *Gammaproteobacteria* (Figure 1.1.11) and, to a lesser degree, also to the *Epsilonproteobacteria*. The high phylogenetic affinity of these bacteria with large groups of free-living bacteria interspersed between symbiotic sequences indicates that chemoautotrophic symbioses have evolved multiple times from different lineages of free-living bacteria (Cavanaugh *et al*. 2005; Stewart *et al*. 2005).

Methanotrophic symbionts form a distinct clade which is closely related to type I methanotrophs. Chemoautotrophic, sulphur-oxidizing symbionts are phylogenetically more diverse, and at present there is evidence for three major clades. Group I contains symbionts from vents and seep vestimentiferan tubeworms as well as symbionts from lucinid, thyasirid, and solemyid clams.

Group II contains mainly symbionts from vesicomyid clams. Symbionts from frenulate tubeworms group into a separate clade.

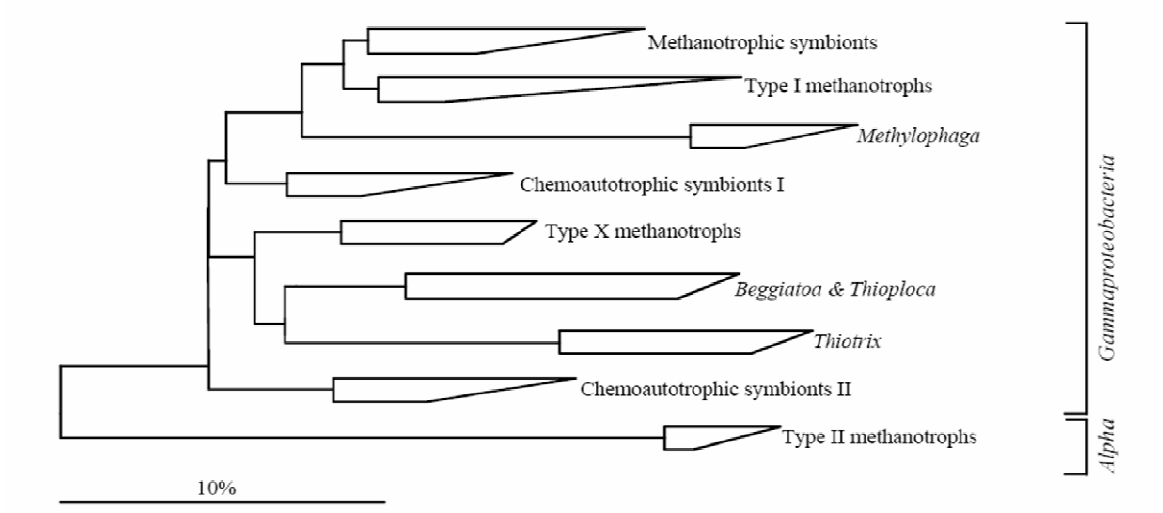

Figure 1.1.11 Phylogenetic tree based on 16S rRNA phylogeny showing placement of free living and symbiotic clades of methanotrophic and sulphur-oxidizing bacteria within the *Gammaproteobacteria*. Type II methanotrophs are used as outgroup and belong to the *Alphaproteobacteria*. From Lösekann (2006)

Chemoautotrophic thiotrophic bacteria oxidize reduced sulphur species such as hydrogen sulphide or thiosulphate and use the energy gained for inorganic carbon fixation (Bright & Giere 2005). One defining characteristic of methanotrophs is the use of enzymes known as methane monooxygenases to catalyze the oxidation of methane to methanol. Methanotrophic bacteria, or methanotrophs, are a division of a physiological group of bacteria known as methylotrophs. Methanotrophs are unique in their ability to utilize methane as a sole carbon and energy source. Methylotrophic bacteria are aerobic bacteria that utilize one-carbon compounds more reduced than formic acid as sources of carbon and energy and assimilate formaldehyde as a major source of cellular carbon (reviewed by Hanson & Hanson 1996).

All autotrophs that fix CO_2 via the Calvin-Benson-Bassham cycle use ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO). Two forms of RubisCO have been found in chemoautotrophic symbionts. For the RubisCO form I, the *ccbL* gene is used as a functional marker, whereas the *cbbM* gene is used for Form II. The gene *aprA* codes for the alpha subunit of adenosine-

5'-phosphosulphate reductase (APS reductase) and is used to characterize thiotrophic bacteria. The APS reductase catalyzed the oxidation of sulphite and AMP to adenosinephosphosulphate and the *aprA* gene has been found in both free-living and symbiotic oxidizers. To characterize methanotrophic bacteria, the genes encoding the particulate and soluble methane monooxygenase (*pmoA*, *mmoX*), and methanol dehydrogenase (*mxaF*) can be used as functional markers (reviewed by Lösekann 2006).

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Chapter 1.2.The Gulf of Cadiz

The Gulf of Cadiz (NE Atlantic) is located between 34ºN and 37º15'N and 6ºW to 9º45'W enclosed by the South Iberian Atlantic Margin and the North African Atlantic Margin of Morocco, west of the Gibraltar Straits (Figure 1.2.1). The bathymetry is steadily increasing from 200m at the shelf edge to depths of over 4000m in the Horseshoe and Seine abyssal plains.

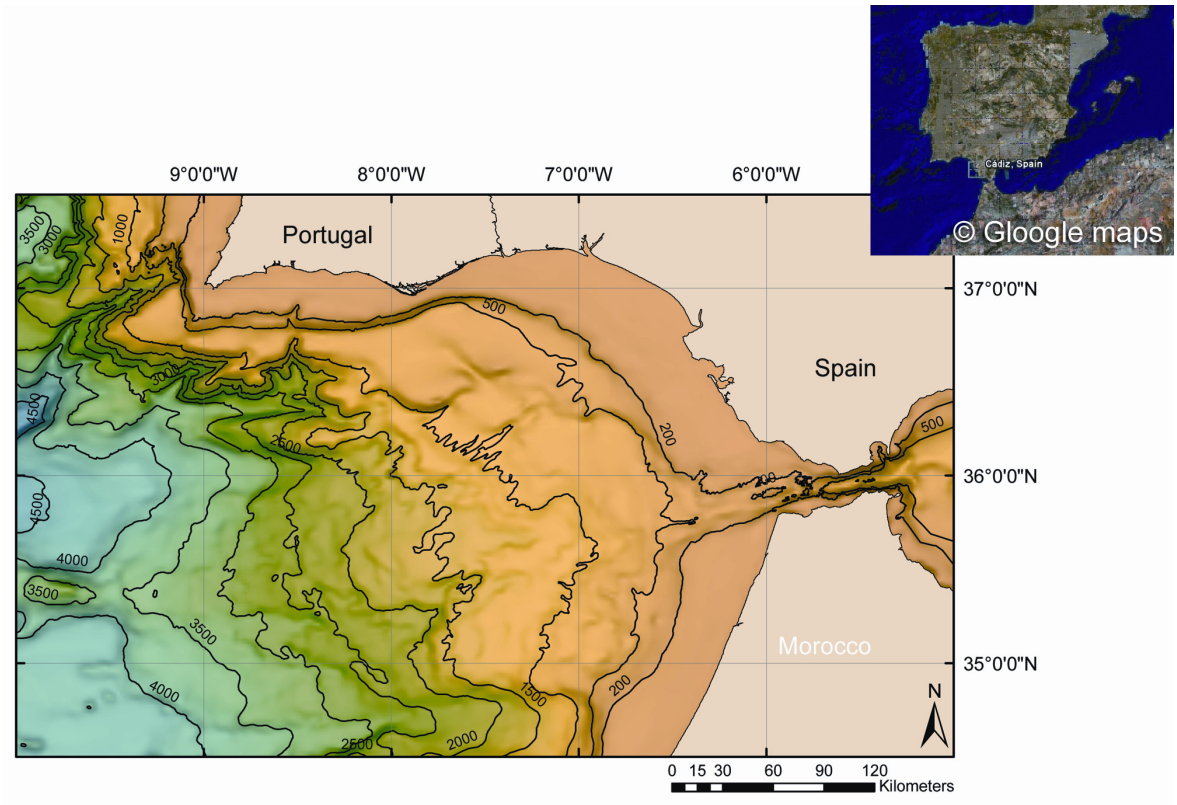

Figure 1.2.1. Geographic location of the study area.

Biogeographically, the Gulf of Cadiz is located in the North East Atlantic warm-temperate zone at the boundary of the Mediterranean Sea zone. Together with the Azores, Madeira and Canary Islands, known by their high biodiversity and level of endemism, West Iberia and the Gulf of Cadiz belong to the Lusitanian province that connects at north to the Biscay region and at south to the Senegal West African region. The Gulf of Cadiz has a privileged location as a biogeographic crossroad where the oceanographic circulation establishes pathways for organism dispersal and favours the links to the Mediterranean, African equatorial and European boreal regions. It is also a key

site to understand important phenomena such as the tectonic evolution of the African-Iberian plate boundary or the influence of water masses (e.g. the Mediterranean Outflow) on seafloor morphology, sedimentation and climate change.

1.2.1.Oceanographic settings

Fully connected to the open ocean, Gulf of Cadiz is influenced by global oceanographic processes such as the seasonal fluctuations of the North Atlantic subtropical gyre. The present day circulation patterns and the hydrographical conditions are dominated by the exchange of water masses through the Strait of Gibraltar

The size and position of the North Atlantic gyre follows the displacements of the Azores atmospheric high, which extends northwards in summer and reduces its size in winter. The pattern of the large scale circulation in the Gulf of Cadiz seems to be anticyclonic in summer with probability to switch to cyclonic in winter. An anticyclonic circulation provides an oceanographic connection between the continental shelf of the northern Gulf of Cadiz (at least the eastern continental shelf) and the Alboran basin in the Mediterranean Sea. The connection is achieved by advection of biogeochemical products residing in the eastern shelf along the northern half of antycyclonic meander that feeds the Atlantic inflow, which is in contact with, or closer to continental waters (Garcia La Fuente & Ruiz 2007).

The Atlantic Ocean at the latitude of the Gulf of Cadiz and off Iberia is characterized by four main water masses (Figure 1.2.2):

1. The Eastern North Atlantic Central Water (ENACW), represented by the descending branch of the Gulf Stream is characterized by temperature values from 8 to 18ºC and salinities between 32.2 and 36.7 (Emery & Meincke 1986); it moves at intermediate depths under the Azores current and circulates in the Gulf of Cadiz between 100 and 850m but its velocity is considered to be significant only bellow depths of about 500m (Fiuza 1984; Fiuza *et al*. 1998).

2. The Mediterranean Water (MW) that corresponds to the Western Mediterranean Intermediate and Bottom Waters is characterized by

temperatures of about 13ºC, high salinities (~36.5), and oxygen content of about 176 µmol.kg^{-1} (Madelain 1970; Ambar & Howe 1979a, 1979b; Ambar *et al*. 2002). The hydrology, morphology and the sedimentary dynamics of the Gulf of Cadiz are intensely modulated by the intermediate water mass of the MW. The horizontal variations of the outflow properties and the complex bottom topography at upper and middle favour the splitting of the plume into several veins (Madelain 1970; Zenk & Armi 1990) and flows are distributed at constant depths along the Cadiz and Guadalquivir channels. As the MW outflow changes the direction from a westward to a north-westward direction due to the Coriolis effect, it is divided into two main cores: the Mediterranean Upper core (MU) and the Mediterranean Lower core (ML) (Madelain 1970; Ambar & Howe 1979a, 1979b; Gardner & Kidd, 1983; Zenk & Armi, 1990). The MU upper core flows between 500 and 800m depth and is characterized by a density of 27.6 (T:~13.4ºC; S:~ 37.07) and an average velocity of 46 cm/s The ML corresponds to the main water flux of the MW in the Gulf of Cadiz. It is centred at about 1200m depth and is characterized by a density of 27.8 (Ambar *et al*. 1999).

3. The North Atlantic Deep Water (NADW) in the Gulf of Cadiz flows southward, underneath the MW, below 2000m depth. The NADW is a multiple-source water mass, characterized by a low stratification and with temperatures ranging from 4-8ºC, salinity values between 24.95 and 35.2 and a with high oxygen content (Emery & Meincke 1986).

4. The bottom water mass in the eastern Atlantic basin is the Antarctic Bottom Water (ABW) that circulates under the NADW at depths greater than 4000m (Emery & Meincke 1986) and that therefore does not have direct influence in the Gulf of Cadiz accretionary wedge region.

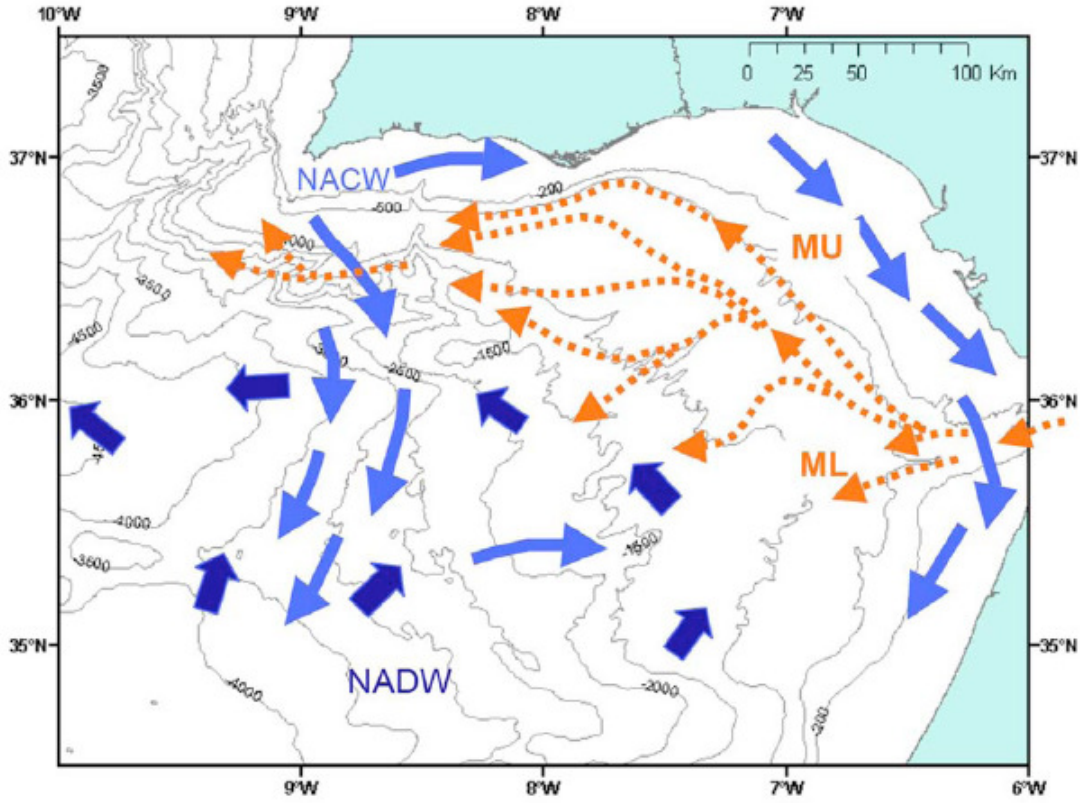

Figure 1.2.2. General circulation patterns in the Gulf of Cadiz. MU: Mediterranean Upper core; NACW: North Atlantic Central Water; NADW: North Atlantic Deep water. Adapted from Hernández-Molina *et al*. (2006)

1.2.2.Geodynamic setting

The Gulf of Cadiz is emplaced in the transition area between the Gloria transform fault zone delineating the African–Eurasian plate boundary in the Atlantic and the Western-most part of the Alpine–Mediterranean orogenic belt (Maldonado *et al*. 1999, Somoza *et al*. 2002; Medialdea *et al*. 2004). This structurally complex convergent tectonic setting includes an accretionary wedge (Gutscher *et al*. 2002) and thick (~7km) Mio-Pliocene sedimentary deposits (Medialdea *et al*. 2004) concealing the boundary between the African and Eurasian plates. The geological history of the area is very complex, entailing several phases of rifting, convergence, and strike-slip motions since the Triassic (Srivastava *et al*. 1990; Maldonado *et al*. 1999; Maldonado & Nelson 1999), intimately related to plate tectonic interaction between

Southern Eurasia and North Africa. There are two major geodynamic driver mechanisms in the area: a) subduction associated with the westward emplacement of the Gibraltar Arc and formation of the Gulf of Cadiz accretionary wedge, probably not active at present and b) oblique lithosphere collision between Iberia and Nubia, active at present and causing active thrusting (Zitellini *et al*. 2009). The regional geodynamic setting is dominated by the slow convergence of 2 to 4 mm/year between the African and Eurasian plates (Argus *et al.* 1989; Sartori *et al.* 1994). The region is deeply faulted and seismically active (Buforn *et al*. 1995; Ribeiro *et al*. 1996). Plate convergence is accommodated along major deeply rooted Paleozoic wrench faults reactivated under Plio-Quaternary transpressive convergence (Meghraoui *et al*. 1996). The formation of the Arc of Gibraltar and expulsion of thick nappes to the west were initiated by a change in the main convergence direction during the mid to Upper-Miocene (Maldonado *et al*. 1999).

In 1992, during the Kane cruise, organized by the Marine Physics Branch of the Naval Research Laboratory, USA, SEAMAP side-scan sonar mosaic and multibeam bathymetry (SEABEAM) were acquired for a large part of the slope, upper and lower continental rise of the central part of the Gulf of Cadiz. This coverage revealed a large number of structures that were inferred to be mud volcanoes (Gardner 2001, 2002). Fluid escape structures were first reported in the shelf break and in the upper continental slope of the eastern-central part of the Gulf of Cadiz in areas covered by muddy sediments (Baraza & Ercilla 1996; Baraza *et al*. 1999). Since then the Gulf of Cadiz has been intensively surveyed with geophysical tools leading to the discovery of the first mud volcanoes, mud diapirs and pockmarks in 1999 (Kenyon *et al*. 2000; Gardner 2001). The TTR cruises carried out under the UNESCO-IOC Training Through Research program played a major role on the discovery and ground-truthing of mud volcanoes in the Gulf of Cadiz (Kenyon *et al*. 2000, 2001, 2002, 2003, 2006; Akhmetzhanov *et al.* 2007, 2008). In addition, several mud volcanoes and diapiric structures covered with carbonate chimneys and crusts were discovered primarily along or in close proximity of the main channels of the Mediterranean outflow water (Kenyon *et al*. 2000, 2001, 2006; Diaz-del-Rio *et al*. 2003; Somoza *et al*. 2003; Kopf *et al*. 2004).

1.2.3.Mud volcanoes and adjacent habitats

It is now well established that the whole area of the Gulf of Cadiz is under compressive deformation and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages.

In the Gulf of Cadiz, the subduction accretionary wedge is covered by a sedimentary pile of variable thickness pierced by mud volcanoes, salt diapirs and other fluid escape features. Mud volcanoes are structurally diverse, ranging in shape from amorphous mud pies to conical structures, and in size from a few meters to kilometers in diameter, attaining heights of up to 100m (Dimitrov 2002). Up to now, over 40 mud volcanoes have been discovered and confirmed by sediment sampling in the Gulf of Cadiz at depths ranging from 230 to 4000m (Kenyon *et al.* 2000, 2001, 2002, 2003, 2006; Pinheiro *et al*. 2003; Akhmetzhanov *et al.* 2007, 2008), The main characteristics of the mud volcanoes in the Gulf of Cadiz were described by Van Rensbergen *et al*. (2005b): they are positive topographic features with diameters up to 5.4km and up to 255m high (e.g. Al Idrisi MV, Figure 1.2.3); the summit may show either a crater depression, a flat top or sometimes a central dome; the flanks are usually irregular with radial outward sediment flows, terraces and/or depositional sediment flow escarpments (lobe-fronts) and in general, a moat surrounds at least part of the base of the mud volcano cones. The moat can be erosive and/or resultant from the collapse of the overburden structure and following the stress release after the eruptions. (Van Rensbergen *et al*. 2005a, 2005b).

Mud volcanism provides an important, but poorly constrained transport pathway for fluids along continental margins. The major mud volcanoes in the Gulf of Cadiz are located either along major strike-slip faults or at the intersection of these faults with arcuate thrusts associated to the formation of the Gibraltar Arc (Pinheiro *et al.* 2003). This fault complex probably acts as a main conduit for the upward-directed transport of deep-sourced overpressurized fluids and hydrocarbon gases. Until present no indications of subsurface accumulations of hydrocarbon were detected during geophysical

surveys although gas hydrates were recovered from Bonjardim, Captain Arutyunov and Ginsburg mud volcanoes (Magalhães 2007).

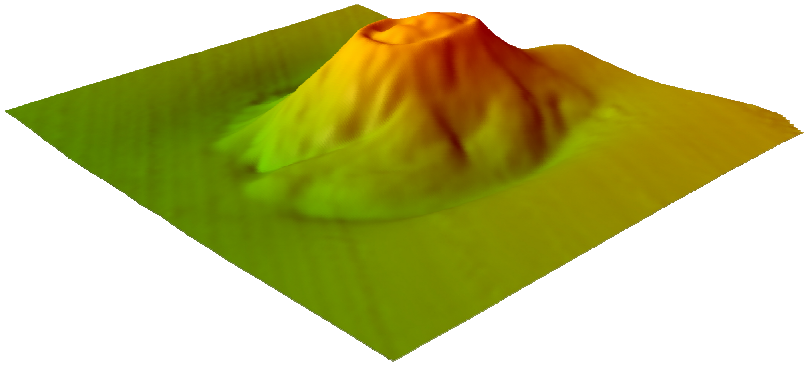

Figure 1.2.3. 3D Model of Al Idrisi Mud Volcano. From Pannemans (2003).

The geochemistry of pore fluids is highly variable at different mud volcanoes and even at different locations of a single mud volcano implying that fluids are transported from different depths/sources through faults which altogether constitute a feeder system (Stadnitskaia *et al*. 2006; Hensen *et al.* 2007). Analysis of the porewater of sediment samples suggests that their chemical composition consists of a mixture of seawater, deeper sourced water of mud volcano fluid, and freshwater from the decomposed gas hydrates (Mazurenko *et al*. 2002). Fluid geochemistry indicates that major fluid formation and overpressuring is caused by clay mineral dehydration at high temperature and several km depths. Very strong enrichments of Li and B suggest high temperature alterations (>150ºC) related to fluid mobilisation along fault systems cutting deeply into the underlying basement (Hensen *et al*. 2007).

Mud breccia sediments in the Gulf of Cadiz are characterized by high gas content, essentially methane that is commonly of thermogenic origin (Nuzzo *et al*. 2005; Stadnitskaia *et al*. 2006) and also high H_2S contents. Compared to other marine gas seeps and methane-rich environments the mud volcanoes in the Gulf of Cadiz show a low or medium range in methane turnover rates, reflecting relatively mild fluxes of methane and sulphide (Niemann *et al*. 2006). Heat-flow measurements from the Gulf of Cadiz were performed during the Anastasya 2001 and SO 175-2 cruise at Faro, Hesperides, Gemini,

Ginsburg, Captain Arutyunov and Bonjardim (Kopf *et al*. 2004). These measurements are affected by the significant seasonal variations of the Mediterranean water that flows close to the sea-floor at 400-1400m depths. Several indications for a slow upward fluid and gas flux were found. Low heat-flow values were recorded in the Captain Arutyunov (17-22 $mW.m^{-2}$), Hesperides (73-100 $mW.m^{-2}$) and Ginsbug mud volcanoes and slightly higher in the Bonjardim (66-163 $mW.m^{-2}$) (Gardner *et al*. 2001; Kopf *et al*. 2004, Hensen *et al*. 2007). The data suggest a complete consumption of methane and higher hydrocarbons in the sediments of the studied mud volcanoes at depths of 20-300cm below the seafloor with no indication of hydrocarbons reaching near-surface sediments or the water column (Hensen *et al*. 2007). However, recent studies located methane and sulphide fronts at 10 to 15 cm in the sediment at Captain Arutyunov mud volcano (Sommer *et al.* 2009).

In some cases microbial processes associated to fluid escape facilitate precipitation of carbonates. The widespread occurrence of such authigenic carbonates in the Gulf of Cadiz is interpreted as an evidence of several episodes of extensive release from the subsurface in the past. Two different types of authigenic carbonates occur in the Gulf of Cadiz: A group dominated by dolomite mineralogy (dolomite crusts, nodules and chimneys) and a group of aragonite dominated carbonates (aragonite pavements, slabs, crusts and buildups). The different morphologic types reflect different geochemical formation environments. The aragonite pavements represent precipitation of authigenic carbonates at the sediment-seawater interface or close to it. The dolomite nodules, crusts and chimneys result from the cementation along fluid conduits inside the sediment column, in more confined geochemical environments (detailed study carried out by Magalhães 2007). The widespread occurrence of authigenic carbonates greatly enhances the habitat heterogeneity in seeps providing hard substrates for the settlement of a variety of benthic organisms including cold water scleractinean corals.

The scleractinean corals are widespread in the Gulf of Cadiz at depths from 200 to 2000m in association to mud volcanoes, mud diapirs and diapiric ridges, but in contrast to the largely flourishing Norwegian and Irish coral reefs, coral growth in the area seems to have ceased and most findings report

on dead thickets and debris of *Dendrophyllia cornigera*, *Dendrophyllia alternata*, *Eguchipsammia cornucopia*, *Madrepora oculata* and *Lophelia pertusa* (reviewed by Wienberg *et al.* in press). Cold water coral-reefs, both living and dead enhace the tridimensional complexity of the benthic habitat and host a rich fauna of invertebrates and fish. The number of species that live on *Lophelia* reefs been estimated to be three times as high as on the soft sediments of the surrounding seabed (Husebo *et al*. 2002).

Four main areas or fields of fluid escape structures have been defined for the Gulf of Cadiz: (1) the Spanish field; (2) the El Arraiche field; (3) the Western Moroccan field; and (4) the deep-water field.

The Spanish field is situated at the middle slope of the northern central part of the Gulf of Cadiz in the Spanish margin. The Mediterranean outflow water, interacting with the rough topography exposes numerous carbonate chimney fields on diapiric ridges and submarine channels (e.g. in the Hormigas, Formosa, Guadalquivir Ridges and Cadiz Channel) but the area also includes several mud volcanoes and mud mounds (e.g. Anastasya, Tarsis, Pipoca, Hesperides, Faro, Cibeles and Almazan).

In the shallow Moroccan margin, the El Arraiche field encompasses Renard Ridge (including Pen Duick Escarpment), Vernadsky Ridge and several large mud volcanoes (e.g. Al Idrisi, Mercator, Fíuza, Kidd, Gemini) located at depths from 230 to approximately 600m depth. The proximity to the euphotic zone and to the African coast adds to the great productivity observed in the area. Dead cold-water scleractinean coral reefs, carbonate crusts and exposed carbonate chimneys characterise the Renard and Vernadsky Ridges. Carbonate crusts, rock blocks and clasts are often found in the craters of the shallow mud volcanoes where mild seepage activity has been recorded (Van Rensbergen *et al*. 2005b). Mercator, one of the shallowest mud volcanoes differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water (Van Rensbergen *et al*. 2005b).

Extensive authigenic carbonate provinces occur at intermediate depths (700-1200m) along the margins of Morocco and Spain. In the Western Moroccan margin the carbonate provinces are accompanied by the frequent occurrence of mounds, thickets and debris of mostly dead cold water

scleractinean corals coral (e.g. around and at the flanks of the Meknès, Student and TTR mud volcanoes). This zone further includes other mud volcanoes (e.g. Yuma, Ginsburg, Jesuz Baraza, Darwin, Chechaouan). The widespread presence of authigenic carbonates suggests that this was a very active seepage area in the past. Darwin mud volcano differs from the others in this area because its crater is completely covered by large carbonate slabs and crusts. Meknès is the southernmost Moroccan mud volcano with a flat crater formed by stiff, sometimes heavily disturbed, green mud breccia rising isolated among an extensive field of small coral mounds.

The deep-water field (1300-4000m), mostly within the Portuguese margin includes several mud volcanoes (e.g. Captain Arutyunov, Carlos Ribeiro, Semenovich, Soloviev, Bonjardim. Porto) that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte 2005). Gas hydrates were recovered from many of these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz (Kenyon *et al*. 2000, 2001, 2002, 2003, 2006; Akhmetzhanov *et al*. 2007, 2008).

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Chapter 1.3.Objectives and Thesis outline

Fluid escape structures were first reported in the Gulf of Cadiz in 1996 and the first evidence of cold seep communities was one living Solemyid bivalve collected in 1999 from Ginsburg mud volcano. Subsequentely the Gulf of Cadiz attracted the attention of many scientists in the fields of Geophysics, Sedimentology, Biogeochemistry and Microbiology among others. It is now well established that the Gulf of Cadiz is an extensive seepage area and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages. However these assemblages remained mostly undescribed and poorly understood.

This thesis intends to improve our knowledge of the biodiversity and functioning of the macrofaunal assemblages of the mud volcanoes and adjacent habitats in the Gulf of Cadiz. The main results are organized in two sections one dedicated to the taxonomy, biodiversity and community structure of the macrofauna (Section 2) and another dedicated to the characterization of trophic relationships and symbiosis (Section 3).

An overview of the biological sampling carried out since 2000 in the Gulf of Cadiz and the current knowledge on the biodiversity and abundance of the macrofaunal assemblages is presented and accompanied by a comprehensive and updated list of species for the region (Chapter 2.1., Annexes 1 and 2) and followed by more detailed work on one of the key taxonomic groups in cold seeps: the Bivalvia. The taxonomic studies of the family Thyasiridae (Chapter 2.2) and of chemosymbiotic species (Chapter 2.3) include the description of new species, notes on biogeographic and bathymetric distribution and a brief ecological discussion. The results on diversity, distribution patterns and species turnover complete the information on the bivalve assemblage (Chapter 2.4). The Section on the biodiversity of macrofaunal assemblages further presents the taxonomy and ecology of the Ophiuroids, one of the most abundant contributors to the deep-sea assemblages yet seldom reported from cold seeps.

The nutritional sources and pathways relevant for the trophic relationships in the benthic foodweb and the importance of chemoautolitotrophic primary

production for the heterotrophic macrofauna is investigated using stable isotopic analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ signatures of key-player species in the mud volcanoes and adjacent habitats in the Gulf of Cadiz (Chapter 3.1). A contribution to the knowledge on symbiosis in cold seeps includes the molecular characterization of microbial symbionts in bivalve (Chapter 3.2) and frenulate hosts (Chapter 3.3).

This Thesis is completed by brief final remarks on the main conclusions and gaps of the biological investigations carried out in the Gulf of Cadiz.

This Thesis includes seven manuscripts, whereof one is already published, two submitted and four in preparation. These manuscripts were prepared in collaboration with different co-authors that greatly contributed to the results presented in this Thesis:

- for the study of bivalves, the taxonomy expert Dr. Graham Oliver from the National Museum of Wales (Cardiff, UK);
- for the study of ophiuroids, the taxonomy expert Dr. Gordon Paterson from the Natural History Musem (London, UK);
- for the molecular work on microbial symbionts, Dr Andrew Weightman and Dr. Gordon Webster that hosted me several times during the internships at Cardiff University (Cardiff, UK).

SECTION 2. MACROBENTHIC ASSEMBLAGES AND TAXONOMY

Chapter 2.1.Macrobenthic community structure at mud volcanoes in the Gulf of Cadiz

Macrobenthic community structure at mud volcanoes in the Gulf of Cadiz

2.1.1.Introduction

Cold seeps are geologically driven ecosystems characterized by the availability of redox couples (e.g. hydrogen sulphide and oxygen, methane and oxygen) that fuel microbial primary production of free-living and symbiotic bacteria and sustain a diverse range of assemblages characterized by the presence of large symbiont-bearing megafauna (mytilid mussels, vesicomyid clams, vestimentiferan and frenulata polychaetes, gastropods or sponges) that typically occur in monospecific and fairly homogeneous patches (Sassen *et al.* 1993; Sibuet & Olu 1998; Sibuet & Olu-Le Roy 2002; Sahling *et al.* 2003; Levin 2005). Special adaptations to cope with toxic environments and the ability to harvest resources from normally unusable chemical compounds are the main competitive advantages of seep species.

A comprehensive account of the present knowledge of the structure of animal communities (abundance, composition, nutrition and behaviour) and how they interact with relevant geochemical and microbial processes is given by Levin (2005) in her review of the ecology of cold seeps. Total macrofaunal densities at seeps may be impoverished (North Sea: Dando *et al.* 1991), enhanced (Santa Barbara: David & Spies 1980; Oregon: Sahling *et al.* 2002; Gulf of Mexico: Levin *et al.* unpublished data), or identical (Levin *et al.* 2003) to those in nearby non-seep sediments. However owing to food availability, higher biomass may occur at seeps compared to the low biomass and high diversity, found in the normal deep-sea (Agard *et al*. 1993).

Cold seeps are therefore “hotspots” of increased biological activity on the seabed. They occur at a variety of spatial scales, from a few meters to several kilometres and are among the most heterogeneous of all continental margin ecosystems. Sources of heterogeneity in cold seeps may arise from local variability in fluid flow, occurrence of gas hydrates, geochemistry, substratum and sediment stability. Carbonate precipitation, a by-product of microbial anaerobic hydrocarbon oxidation, and biogenic habitats created by foundation

invertebrate species (symbiont-bearing, sponges, soft and stony corals) provide substrata for a variety of macrofaunal and meiofaunal assemblages (Cordes *et al.* in press). These faunal assemblages respond to changes in structural complexity, habitat geochemistry, nutrient sources, and biotic interactions.

The Gulf of Cadiz has a strategic central location in relation to other known seeps (Nordic margin, Western Mediterranean, Gulf of Guinea) and vents (Mid-Atlantic Ridge). Hydrological factors interacting with the complex topography and geological setting of the Gulf of Cadiz, produce a considerable environmental heterogeneity that is expressed by the occurrence of a rich and diverse range of habitats (comprising mud volcanoes, diapiric ridges, carbonate chimney fields, cold water coral reefs) and their associated macrofaunal assemblages. The knowledge on cold seep communities in this region may be of utmost importance for understanding dispersal of chemosynthetic organisms and connectivity among cold seeps and between cold seep and vent habitats.

In this chapter we provide an overview of the biological sampling carried out since 2000 in the Gulf of Cadiz under the frame work of the Training Through Research programme (UNESCO-IOC) and the HERMES project (7FP-EU). Although samples from the most recent cruises are still being processed, the current knowledge on the biodiversity and abundance of the macrofauna from the cold seeps in the Gulf of Cadiz is presented.

2.1.2.Material and methods

2.1.2.1.Study sites and sample collection

The study sites include twenty mud volcanoes and other eleven locations from adjacent habitats (carbonate mounds and cold-water coral reefs) covering a depth range of 230 to 3900m. (Figure 2.1.1, Annex I).

The biological dataset from the Gulf of Cadiz was obtained during several cruises (TTR 10 to TTR12, TTR14 to TTR17, MSM01-03, JC10 and 64PE284) carried out from 2000 to 2008 and include a series of samples taken with a diverse array of quantitative and non-quantitative sampling gear (Annex I,

Figure 2.1.2, Table 2.1.1): box-corers (USNEL and NIOZ), TV-grabs (two-spade and orange peel) and multicorers (MGC). Samples were also recovered from lander operations (BIGO and FLUFO chambers) and ROV dives (suction, scoop andd push-cores). Geological dredge samples and rocks collected during ROV dives provide some specimens of mostly epibenthic sessile organisms.

The USNEL Box-corer is a standard quantitative sampler of deep-sea macrofauna. It consists on a box covering an area of 50x50cm with a side panel than can be removed enabling photographing, description and subsampling of the sediment layers. The Multicorer (MUC) is used to provide simultaneously up to 8 seafloor replicate samples in individual cores of 10cm diameter.

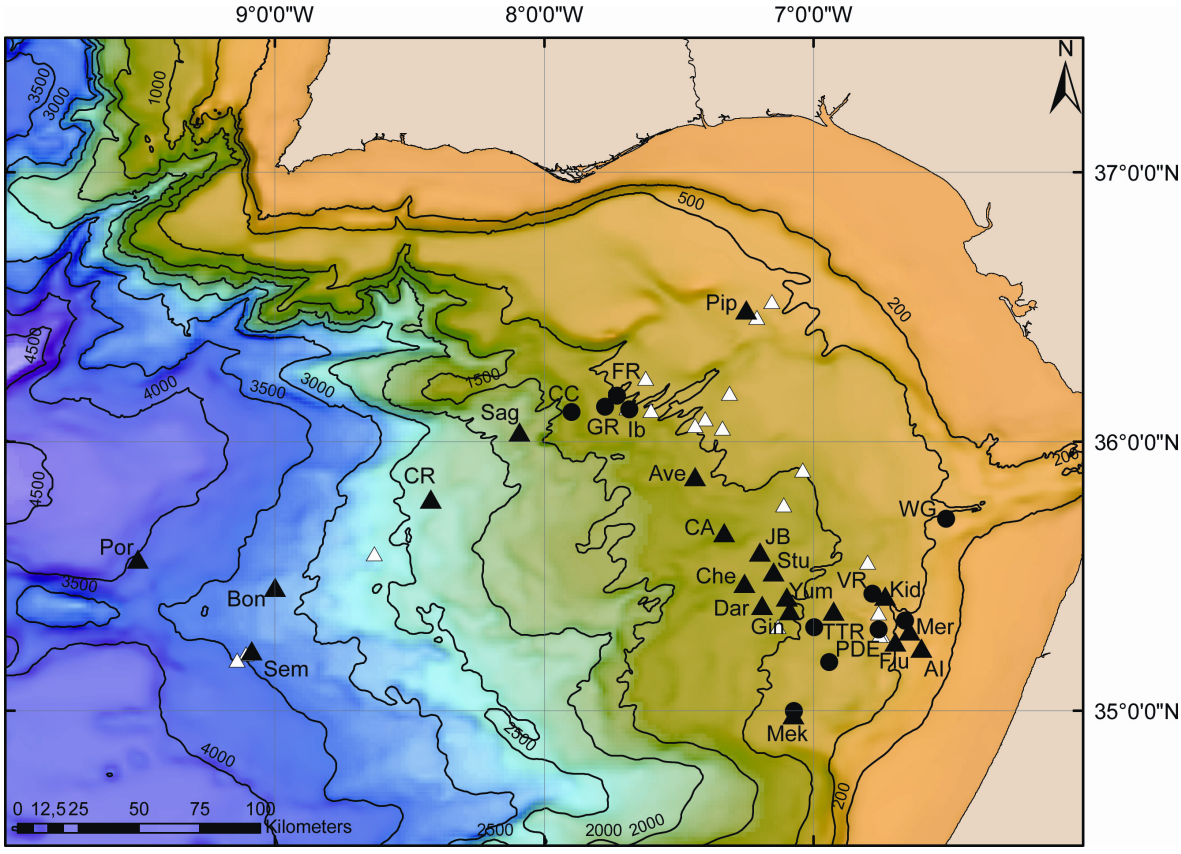

Figure 2.1.1. Location of the different study sites. AI: Al Idrisi, Ave: Aveiro, Bon: Bonjardim, CA: Captain Arutyunov, Che: Chechouen, CR: Carlos Ribeiro,Dar: Darwin, Fiu: Fiuza, Gem: Gemini, Gin: Ginsburg, JB: Jezus Baraza, Kid: Kidd, Mek: Meknès, Mer: Mercator, Ole: Olenin, Por: Porto, Rab: Rabat, Stu: Student, Tan: Tanger, Tas: Tasyo, TTR and Yum: Yuma mud volcanoes.

Two different TV-controlled grabs were used both operating up to 6000m water depth: the DG-1 Grab system, is a 1500kg system with a maximum interior volume of 0.4m^3; the GTVD-2 is an orange peel grab has 3.2 tons and an interior volume of 1.1m^3. Both systems have a video camera fitted, and power and light units, allowing real time visualization of the seafloor during the grab operation. The onboard unit allows the visualization and record of the video images for selection of the sampling site. Underwater positioning and navigation of the TV-grab is registered by the underwater navigation system.

The chambers of BIGO and FLUFO landers (IFM-GEOMAR, Kiel) also provide good samples for macrofauna. The landers are deployed using a video-guided launcher. The BIGO lander is a biogeochemical observatory that can be used to study the oceanic benthic boundary layer in situ. BIGO simulates different environmental conditions (oxygen content/organic influx) *in situ* and represents an ideal experimental platform to study the kinetics of prominent biogeochemical reactions and threshold levels involved in the regulation of seabed methane emission. The FLUFO lander is a fluid flux observatory for the quantification of aqueous and gaseous fluxes and their control parameters.

A complete list of stations with information on location, depth and sampler is given in Annex I

Table 2.1.1. List of gears used during the several cruises. Area sampled is given for quantitative samples.

Cruise	Gear	Abbreviation	Area sampled (m^2)	Main target
TTR	Dredge	D	—	Bio/Geological
	Gravity Corer	G	—	Geological
	Kasten Corer	K	—	Geological
	TvGrab	GR	—	Bio/Geological
	USNEL-Box Corer	B	0.25	Biological
MSMERIAN	Biogeochemical Observatory	BIGO	0.07	Geochemical
	Fluidflux observatory	FLUFO	0.07	Geochemical
	Multicorer	MUC	0.02	Geochemical
	USNEL-Box Corer	GKG	0.25	Biological

2.1.2.2.Sample processing

In all cruises the sample processing was initated onboard with different procedures according to the specific samplers.

Non-quantitative samples. *TvGrab samples*: Conspicuous animals were picked from the surface of the sediments and rocks. When chimneys and crusts were present, specimens were also recovered from their washings (the finer sieve used was 0.5 mm). A variable volume of surficial sediment was also collected when available and washed through a sieve column (2.0, 1.0 and 0.5 mm).

Dredge samples and ROV rock samples: the fauna was picked from the surface of the rocks and/or recoved from rock washings and sieved sediments (the finer sieve used was 0.5 mm).

ROV suction and scoop samples: the whole sample was kept.

Quantitative samples. *Box-corers, multi-cores and push-cores:* Sub-sampling of the different depth layers of the sediment was carried out whenever possible. The material was sliced at 0-1, 1-3, 3-5, 5-10, 10-15cm and whenever possible also 15-20cm following the standard techniques published by the Census of Marine Life workshop on the study of "Biodiversity of Deep-sea Sediments" and adopted as general practice in HERMES. The overlying water was sieved through a 0.5mm sieve in order to retrieve any small swimming fauna. For multi-corer samples, individual cores were processed separately. All sediment layers were carefully washed with seawater through a sieve column (2.0, 1.0 and 0.5mm).

Lander chambers: whenever possible the whole content of the chamber was carefully washed with seawater through a sieve column (2.0, 1.0 and 0.5mm); in some cases subsampling for different purposes was carried out prior to washing.

Preservation. The washed sediment samples in the two coarser fractions (2 and 1 mm) were kept in 96% ethanol. The finer fraction (0.5 to 1.0 mm) of the sieved sediments was preserved in 10% buffered formalin diluated in seawater and stained with Rose Bengal. The specimens picked from sediments and rocks were preserved in 96% ethanol (or deep-frozen) to enable genetic analysis. Some specimens of selected species were prepared and deep-frozen for stable isotope analyses or fixed in a solution of gluteraldehyde for endosymbiont analysis.

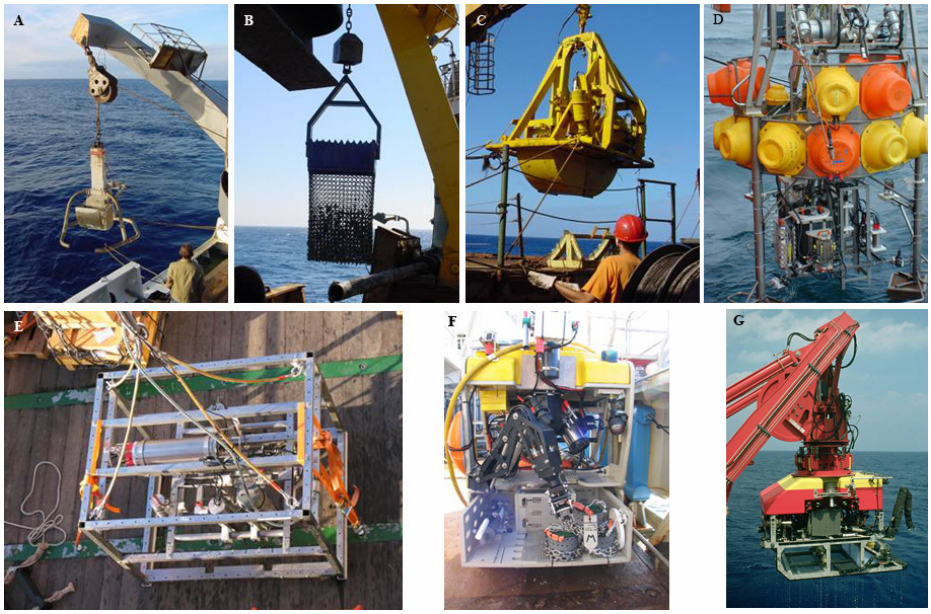

Figure 2.1.2. Examples of the variety of sampling gears used during the sampling of macrofauna in the Gulf of Cadiz. A: USNEL Boxcore (MSU), B: Geological Dredge (MSU), C: Two-spade TV assisted Grab (MSU), D: BIGO lander (IFM-GEOMAR), E: OFOS (deep-towed video observation system, IFM-GEOMAR) F: ROV Cherokee (MARUM & Gent Univesity), G: ROV Isis (NERC & NOCS).

Identification. The specimens were all sorted into major taxa (Class, Order). The taxonomical work was carried out in CESAM at the University of Aveiro and in collaboration with several specialists from other institutions (listed in Annex II).

Although many specimens were not yet ascribed a species name they are all sorted into different operational taxonomic units (OTUs) and ascribed a codename consistent throughout the samples. Most specimens are held in the Biological Research Collection of the University of Aveiro (Department of Biology) and will be available for further ecologic, taxonomic, morphologic and genetic studies. Holotypes and paratypes of new species were deposited in adequate Museums and registered permanent collections.

2.1.2.3.Density and biodiversity

Density and biodiversity of macroinvertebrates was assessed for the samples collected during the cruises TTR10, TTR11, TTR12, TTR14, TTR15, TTR16 and MSM01-03. Samples taken in 2007 (cruise JC10) and 2008 (cruises 64PE284 and TTR17) are currently being processed and therefore will not be considered for the results presented herein.

Because the taxonomical work is still in progress, biodiversity is expressed generally as "number of taxa". Species richness, Hulbert´s expected species richness ($ES_{(n)}$), Shannon-Wiener diversity index (H') and Pielou's evenness index (J') were calculated using the community analysis program PRIMER (Clarke & Warwick 2001) for quantitative samples obtained from box-cores. Rarefaction curves (Gotelli & Colwell 2001) were plotted based in the same samples. When several samples were available for the same mud volcano these were pooled in order to originate a single curve for each mud volcano. Owing to the qualitative nature of most of the samples and the generally low number of samples taken for several mud volcanoes comparative analyses are not possible and therefore the results are presented in a rather descriptive mode. The total dataset of taxa present in 64 samples from 17 mud volcanoes was used to represent the regional biodiversity of the macrofauna, shown herein using accumulation, loss and turnover curves plotted against increasing depth (Colwell *et al*. 2004; Hurlbert 1971; Ugland *et al.* 2003).

For density estimates modular organisms (e.g. Porifera, Cnidaria, Bryozoa) were always excluded. Density (ind.m^{-2}) was calculated only for box-corer (BC) replicates and therefore restricted to a small number of samples from seven mud volcanoes: Mercator (3 BC collected during TTR15 and 2 BC taken during MSM01-03), Kidd (3 BC, TTR14), Meknès (2 BC, MSM01-03), Captain Arutyunov (2 BC, MSM01-03), Carlos Ribeiro (1 BC, MSM01-03), Bonjardim (1 BC, MSM01-03) and Porto (1 BC, MSM01-03). The values presented herein do not account for Siboglinidae densities: in most samples siboglinids were very fragmented and the time-consuming task of identifying countable individuals is not yet completed (only the anterior part of the body with head and tentacle(s) should be counted as one individual).

2.1.3.Results

2.1.3.1.Taxonomy

A detailed taxonomic list with the lowest possible taxonomic level possible at the moment is provided in Annex II. For consistency, the taxonomic classification is presented according to the World Register of Marine Species

(www.marinespecies.org). More than 14550 specimens were examined and ascribed to different taxonomic groups. Arthropoda (7279 ind.) and Anellida (3000 ind. excluding siboglinids) were the most abundant Phyla, followed by Mollusca (1415 ind.). Much less common were the Echinodermata (481 ind.) and finally the Sipuncula (254 ind.). Arthropoda (with 199 taxa of Crustacea) and Anellida (141 taxa of Polychaeta identified to species or genus level) are the most speciose groups followed by Mollusca and Cnidaria. The taxonomic study of Hydrozoa (49 species), Bivalvia (50 species), Decapoda (18 species) and Ophiuroidea (13 species) has already been carried out with great detail by specialists in these groups. In other groups (like Porifera, Anthozoa, Polychaeta) only part of the material has been examined by specialists and in the case of Gastropoda all the specimens were ascribed to putative taxa without the help of a specialist.

New species. The detailed examination of the collected specimens allowed the identification of numerous undescribed species. Some of these species (listed below and pictured in Figure 2.1.3) were described and are already published (5 species) or prepared for publication (6 species).

Phylum Cnidaria;

Class Anthozoa; Order Alcyonacea; Family Dendrobrachiidae

Dendrobrachia sp. nov. López-González, Zibrowius & Cunha, in prep.

Class Hydrozoa; Order Anthoathecata; Family Tibiclavoidae

Tublicavoides striatum Moura, Cunha & Schuchert, 2007

Phylum Mollusca

Class Bivalvia; Sub-Class Heterodonta; Family Lucinidae

***Lucinoma* sp.** nov. Oliver, Rodrigues & Cunha, in prep. (Chapter 2.3)

Class Bivalvia; Sub-Class Heterodonta; Family Thyasiridae

Thyasira vulcolutre Rodrigues & Oliver, 2008 in Rodrigues *et al.* 2008 (Chapter 2.2)

Class Bivalvia; Sub-Class Heterodonta; Family Vesicomyidae

Isorropodon sp. nov. Oliver, Rodrigues & Cunha, in prep. (Chapter 2.3)

Class Bivalvia; Sub-Class Protobranchia; Family Solemyidae

Acharax sp. nov. Oliver, Rodrigues & Cunha, in prep. (Chapter 2.3)

Petrasma sp. nov. Oliver, Rodrigues & Cunha, in prep. (Chapter 2.3)

Phylum Anellida

Class Polychaeta; Order Sabellida; Family Siboglinidae

Bobmarleya gadensis Hilário & Cunha, 2008

Spirobrachia tripeira Hilário & Cunha, 2008

Phylum Arthropoda

Class Malacostraca; Order Decapoda; Family Calianassidae

Vulcanocalliax arutyunovi Dworshack & Cunha, 2007

Phylum Echinodermata

Class Stelleroidea; Order Ophiurida; Family Ophiacanthidae

Ophiopristis cadiza Rodrigues, Paterson, Cabrinovic & Cunha, submitted (Chapter 2.4)

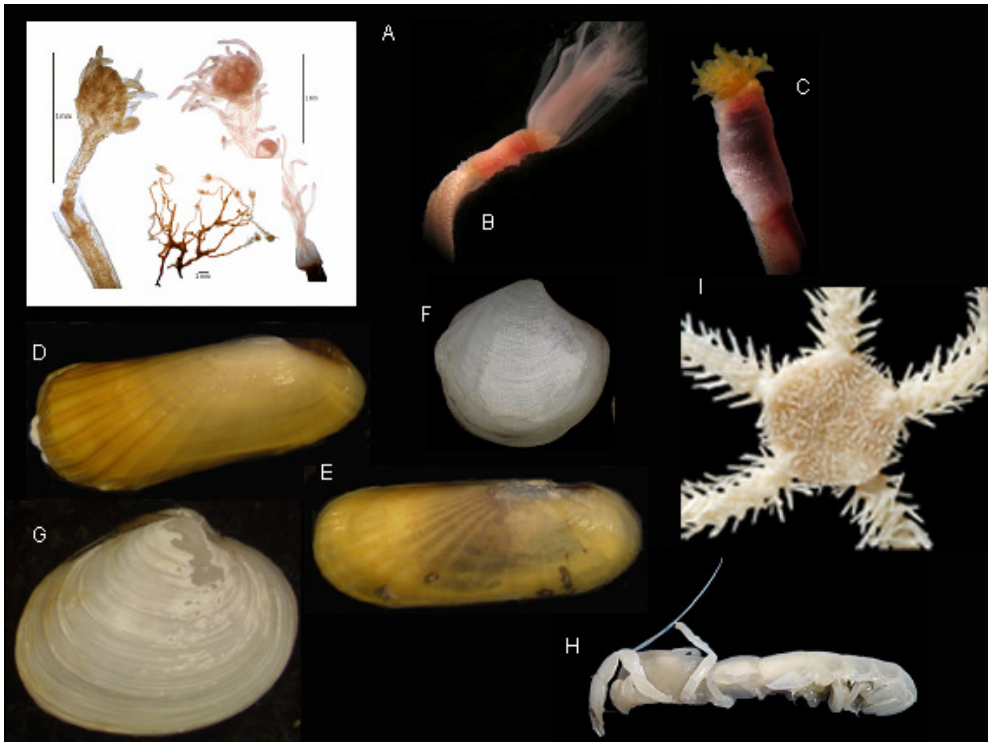

Figure 2.1.3. Some of the new species described for the Gulf of Cadiz: A. *Tublicavoides striatum*; B: *Spirobrachia tripeira*; C: *Bobmarleya gadensis*; D: *Acharax* sp. nov.; E: *Petrasma* sp. nov.; F: *Thyasira vulcolutre*; G: *Isorropodon* sp. nov. ; H: *Vulcanocalliax arutyunovi*; I: *Ophiopristis cadiza*.

2.1.3.2.Biodiversity and distribution of major taxa

A summary of the provisional number of macroinvertebrate taxa found in the mud volcanoes and adjacent habitats from the Gulf of Cadiz is shown in Table 2.1.2. Taxa richness is given for the major taxonomic groups but these provisional values are conservative estimates as only 507 taxa were identified to species or genus level and each one of the 136 "undetermined" may in fact represent more than one species. Additionally several species complexes were detected in 4 hydrozoan taxa; these are composed by a still uncertain number of cryptic species (C. Moura pers. comm.).

Table 2.1.2. Summary of the provisional taxa richness of major taxonomic groups in the macroinvertebrate assemblage of mud volcanoes and adjacent habitats in the Gulf of Cadiz. The values under "Determined" are the number of taxa identified to species or genus level. The values under "Undetermined" and "Cryptic" are conservative as the identification of the respective taxa is still undergoing.

Taxa	Determined	Cryptic	Undetermined	Total
Porifera	26		2+	**28+**
Hexactinellida	(4)		(1+)	
Demospongiae	(22)		(1+)	
Cnidaria	74	4+	6+	**84+**
Anthozoa	(24)		(4+)	
Hydrozoa	(49)	(4+)	(2+)	
Scyphozoa	(1)			
Mollusca	50		40+	**90+**
Gastropoda			(37+)	
Bivalvia	(50)			
Other			(3+)	
Anellida	141		54+	**195+**
Polychaeta				
Scolecida	(17)		(16+)	
Aciculata	(58)		(9+)	
Canalipalpata	(66)		(28+)	
Other			(1+)	
Arthhropoda	199		24+	**223+**
Decapoda	(18)			
Amphipoda	(83)		(4+)	
Cumacea	(16)		(4+)	
Isopoda	(58)		(4+)	
Tanaidacea	(19)		(10+)	
Other	(5)		(2+)	
Echinodermata	16		4+	**20**
Crinoidea	(1)			
Echinoidea	(2)		(1+)	
Stelleroidea				
Asteroidea			(3+)	
Ophiuroidea	(13)			
Other	1		6+	**7+**
TOTAL	**507**	**4+**	**136+**	**647+**

From the 20 mud volcanoes and 11 other sites in adjacent habitats sampled up to now in the Gulf of Cadiz, this thesis focus on the biodiversity of 17 mud volcanoes based on samples taken before 2007: Al Idrisi, Mercator, Fiúza, Kidd, TTR, Meknès, Student, Yuma, Ginsburg, Aveiro, Jesuz Baraza, Darwin, Chechouan, Captain Arutyunov, Carlos Ribeiro, Bonjardim and Porto.

As mentioned before these sites were sampled using a diverse array of sampling equipment but in most sites only one to three samples were taken. The species richness and composition of samples taken in Mercator mud volcano using different samplers (Figure 2.1.4) clearly show the high variability of the sampled assemblage.

Although there is a high variability that can be related to the environmental heterogeneity within the mud volcano (see for instance the differences in species number and composition of the different boxcore samples) there are also important differences among samples taken with different samplers. The number of taxa yielded in each sample is clearly dependent on the size of the sampler: quantitative samplers with smaller sampling areas yield lower taxa richness (megacore vs box core). Samplers may also sample selectively different taxonomic or functional groups: dredge samples yield very few arthropod and mollusc species but are very important to sample sessile groups such as sponges and cnidarians; deeply buried or large animals are frequently collected with a TV-grab but are seldom represented in megacore or even boxcore samples.

The taxa richness of these mud volcanoes arranged from shallower to greater depths is presented in Figure 2.1.5 showing an apparent trend of decreased taxa richness with increasing depth. The data shown represents the total accumulated number of species from all samples (both quantitative and qualitative) collected from each mud volcano. Consequently the values are biased by the different sampling effort dedicated to each site and the best sampled mud volcanoes (Mercator, Kidd, Meknès and Captain Arutyunov) are also the ones that show the greatest taxa richness. The number of taxa varied from less than 20, in sites with a single sample (Al Idrisi, Student, Aveiro), to values one order of magnitude higher, in well-sampled sites (Captain Arutyunov: 190 taxa; Mercator: 273 taxa).

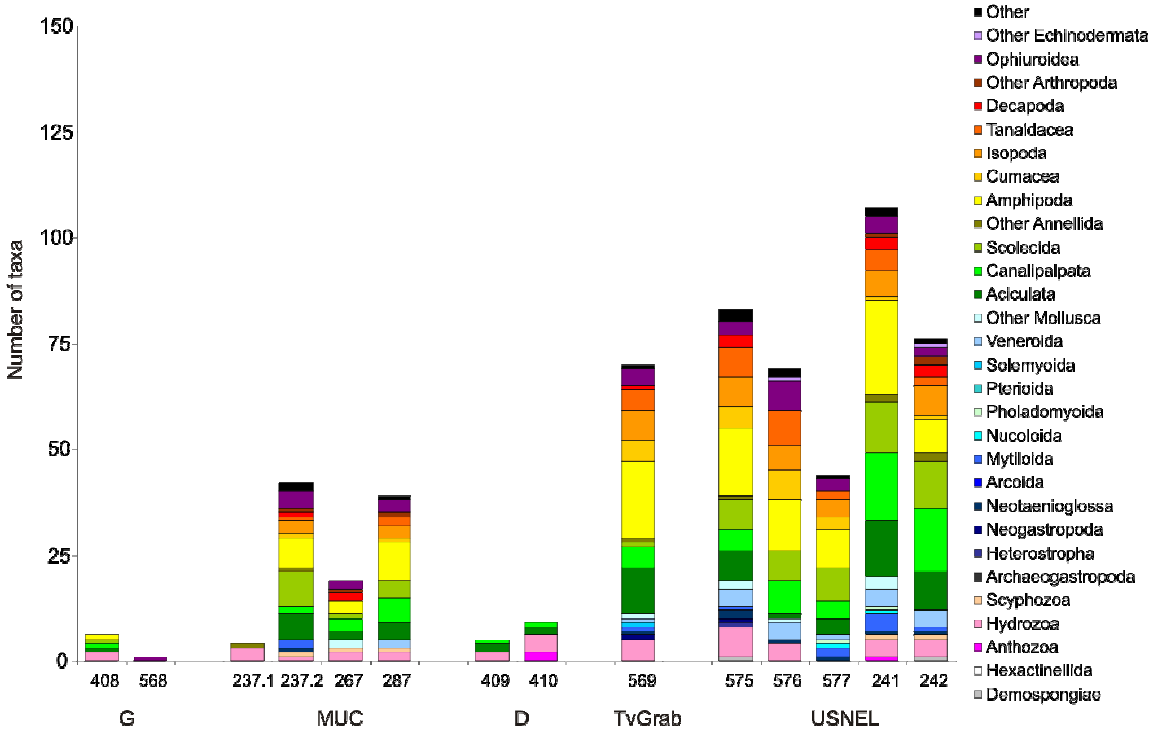

Figure 2.1.4. Taxa richness in samples taken in Mercator MV using different sampling gear. Numbers are stations numbers according to Table 1, Annex I. G: Gravity corer, MUC: Multicorer, D: Dredge, TvGrab: Tv-assisted Grab; USNEL: USNEL Box-corer.

It is also evident that there is a great variability in the species composition of the macroinvertebrate assemblage of the different mud volcanoes. A brief account on the distribution and species richness of the major taxonomic groups is given below. For some of these groups detailed information is shown in figures where the mud volcanoes (the three mud volcanoes with the lowest species richness were excluded: Al Idrisi, Student and Aveiro) are arranged in the three main regional groups: the shallow Moroccan area, the Western Moroccan area and the Portuguese deep-water area.

Cnidaria. Cnidarians represented by over 84 taxa sampled at all depths but were most frequent in the shallower areas (below 1200m). They occurred frequently in mud volcanoes where hard-substrata were available but mostly in the adjacent habitats (cold-water corals and carbonate concretions). Detailed work on hydrozoan taxonomy and genetic diversity is being currently carried out by by C. Moura (e.g. Moura *et al*. 2007, 2008).

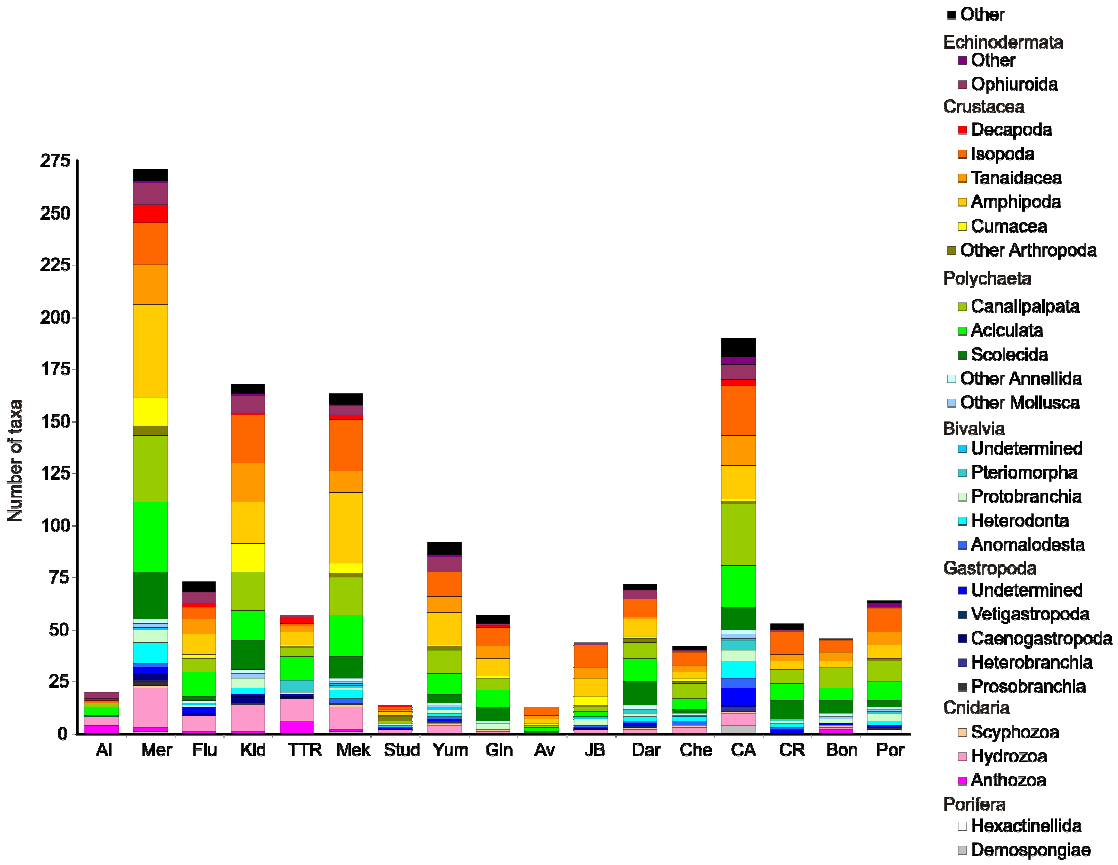

Figure 2.1.5. Taxa richness in the 17 mud volcanoes studied from the Gulf of Cadiz. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). AI: Al Idrisi; Mer: Mercator; Fiu: Fiúza; Kid: Kidd; Mek: Meknès; Stud: Student; Yum: Yuma; Gin: Ginsburg; Av: Aveiro; JB: Jesuz Baraza; Dar: Darwin; Che: Chechouan; CA: Captain Aruryunov; CR: Carlos Ribeiro; Bon: Bonjardim; Por: Porto.

Mollusca. Molluscs were represented by 1415 individuals ascribed to over 90 taxa. The highest molluscan diversity was observed in Captain Arutyunov (35 species) and Mercator (28 species). The general trend, both for Bivalvia and Gastropoda, is for a higher diversity in the shallower Moroccan and deep-water Portuguese fields and lower diversity in the Western Moroccan field (Figure 2.1.6, Figure 2.1.7). The distribution of bivalves is given in detail in Chapters 2.2, 2.3 and 2.4. The Neogastropoda were only found in the shallower mud volcanoes but the high number of undetermined gastropod species difficults the detection of other distributional patterns.

Anellida. Anellids were ascribed to over 195 polychaete taxa. The total number of specimens collected is not yet determined as the counting of siboglinids is not completed, the other polychaete families sum up to 3000 individuals. The three main polychaete groups, Aciculata, Canalipalpata and Scolecida were well-represented in all mud volcanoes (Figure 2.1.8). The Canalipalpata are predominantly small sized, low mobility, surface and subsurface deposit feeders. They were the most speciose group (over 94 different taxa) except in some mud volcanoes of the western Moroccan field, likely owing to the widespread occurrence of carbonate crusts covering the soft sediments in this area. The Aciculata were also well represented (over 67 different taxa) in most mud volcanoes, especially in the shallower ones; these polychaetes are frequently large-sized, mobile carnivores and omnivores that may prey upon small crustaceans and other polychaetes that are very abundant in the mud volcano sediments.

The most frequent families were the Sibloginidae specimens appeared on 15 of the 17 mud volcanoes studied), Glyceridae, Lumbriniridae and Paraonidae (13 mud volcanoes) and Onuphidae and Ampharetidae (12 mud volcanoes).

Arthropoda. Arthropods were the most speciose group with 7279 individuals ascribed to over 223 taxa. From the 18 species of decapods, mostly collected from the shallower mud volcanoes, only the crab *Monodaeus couchi* was frequently sampled in several mud volcanoes and the majority of species occurred as singletons. Most arthropods were represented by peracarid crustaceans (Figure 2.1.9). Amphipods were the most speciose but their species richness markedly decreased toward greater depths. The same pattern was observed for cumaceans that were not found at depths greater than 2000m. Tanaids and especially isopods maintained a more constant number of taxa and therefore reinforced their relative contribution in the deeper benthic assemblages.

The analysis of the arthropoda on the 17 mud volcanoes studied revealed that there is a contribution of 35.3, 29.4, 28.6, 4.3, 1.2 and 1.2% for tanaids, amphipods, isopods, cumaceans, decapods and others respectively.

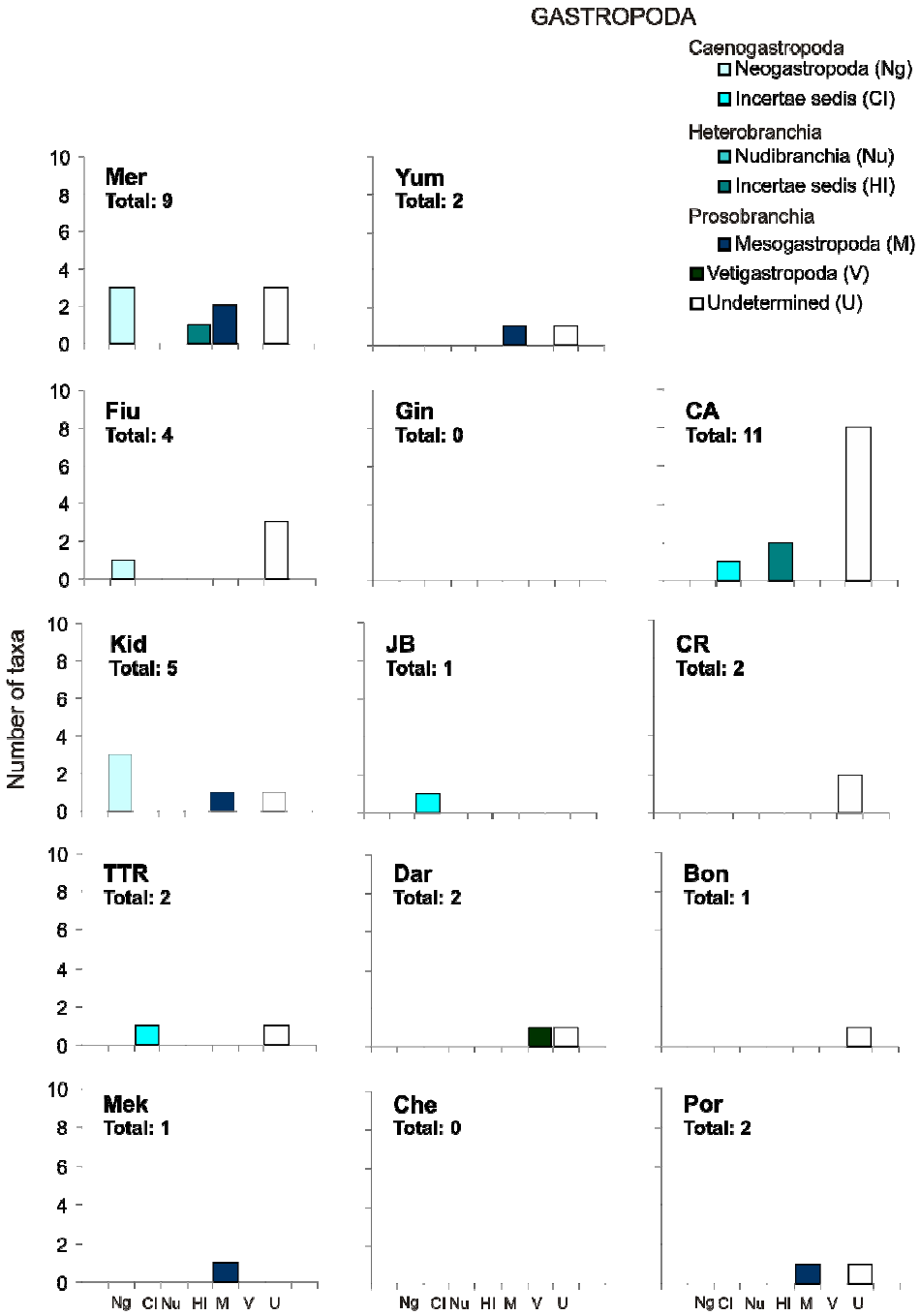

Figure 2.1.6. Taxa richness of different groups of Gastropoda in the different mud volcanoes studied. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). The shallow Moroccan field includes Mercator (Mer), Fiúza (Fiu) and Kidd (Kid); the Western Moroccan field includes TTR, Meknès (Mek), Yuma (Yum), Ginsburg (Gin), Jesuz Baraza (JB) Darwin (Dar), Chechouan (Che) and the deep-water field includes Captain Aruryunov (CA) Carlos Ribeiro (CR), Bonjardim (Bon) and Porto (Por).

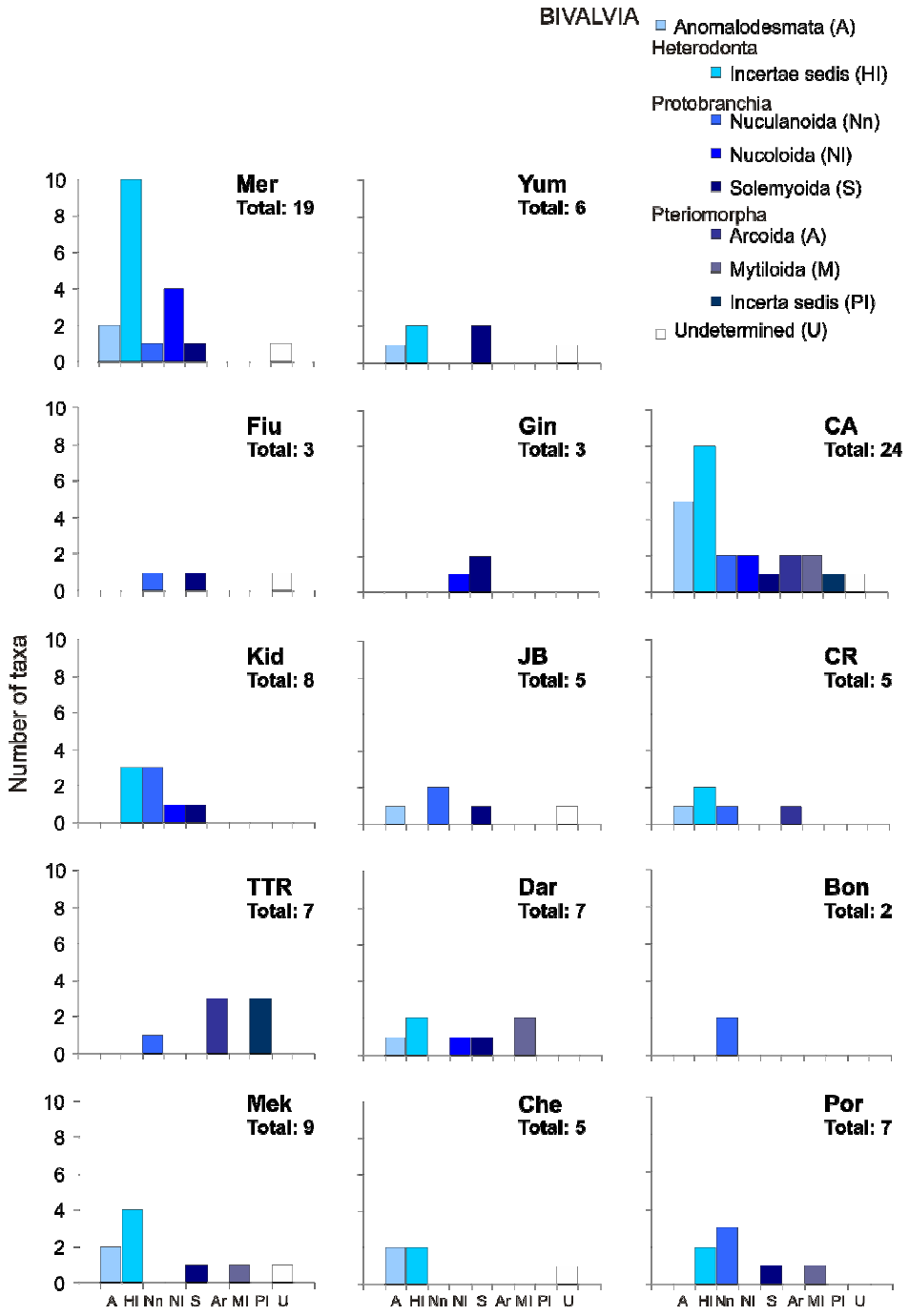

Figure 2.1.7. Taxa richness of different groups of Bivalvia in the different mud volcanoes studied. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). The shallow Moroccan field includes Mercator (Mer), Fiúza (Fiu) and Kidd (Kid); the Western Moroccan field includes TTR, Meknès (Mek), Yuma (Yum), Ginsburg (Gin), Jesuz Baraza (JB) Darwin (Dar), Chechouan (Che) and the deep-water field includes Captain Aruryunov (CA) Carlos Ribeiro (CR), Bonjardim (Bon) and Porto (Por).

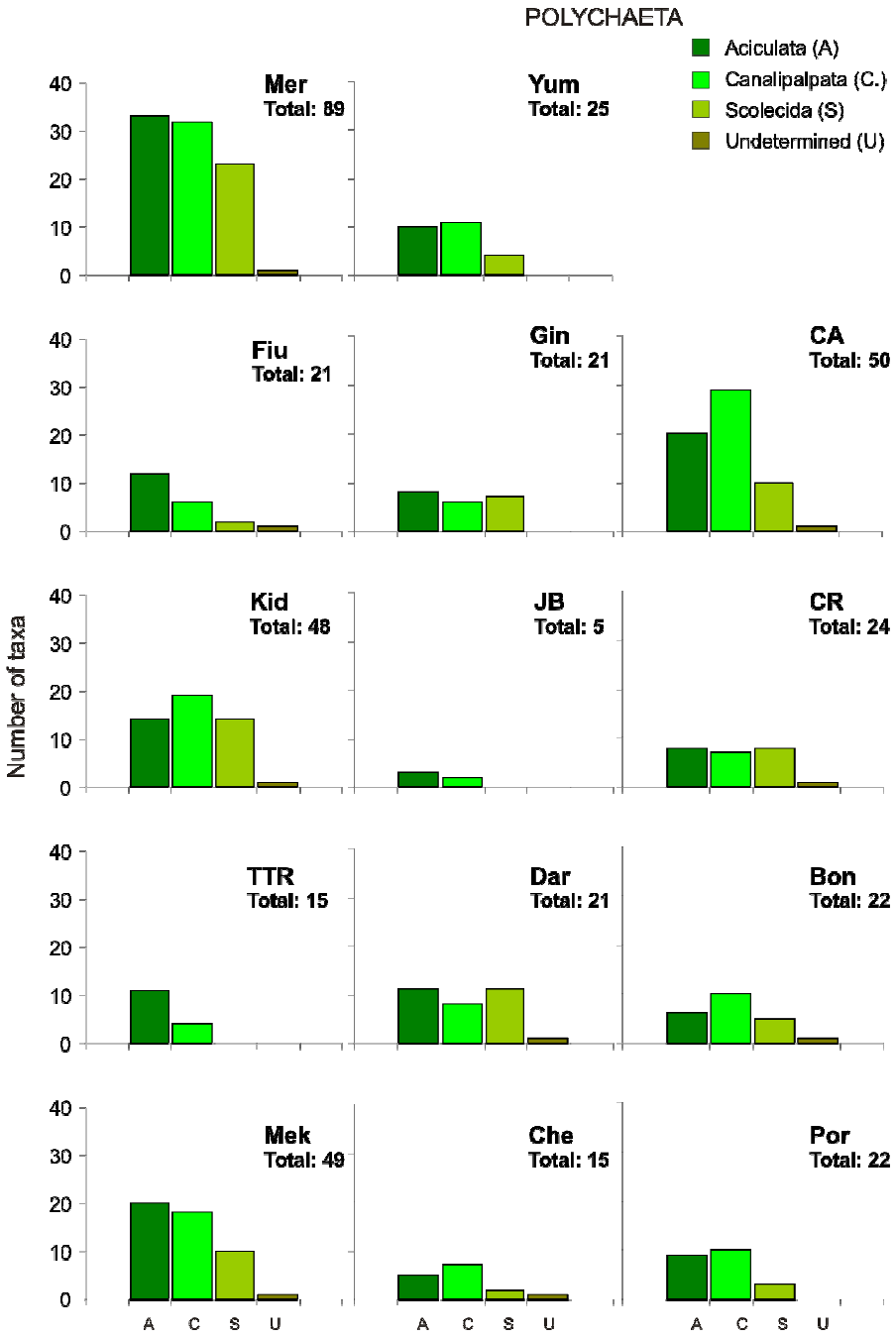

Figure 2.1.8. Taxa richness of different groups of Polychaeta in the different mud volcanoes studied. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). The shallow Moroccan field includes Mercator (Mer), Fiúza (Fiu) and Kidd (Kid); the Western Moroccan field includes TTR, Meknès (Mek), Yuma (Yum), Ginsburg (Gin), Jesuz Baraza (JB) Darwin (Dar), Chechouan (Che) and the deep-water field includes Captain Aruryunov (CA) Carlos Ribeiro (CR), Bonjardim (Bon) and Porto (Por).

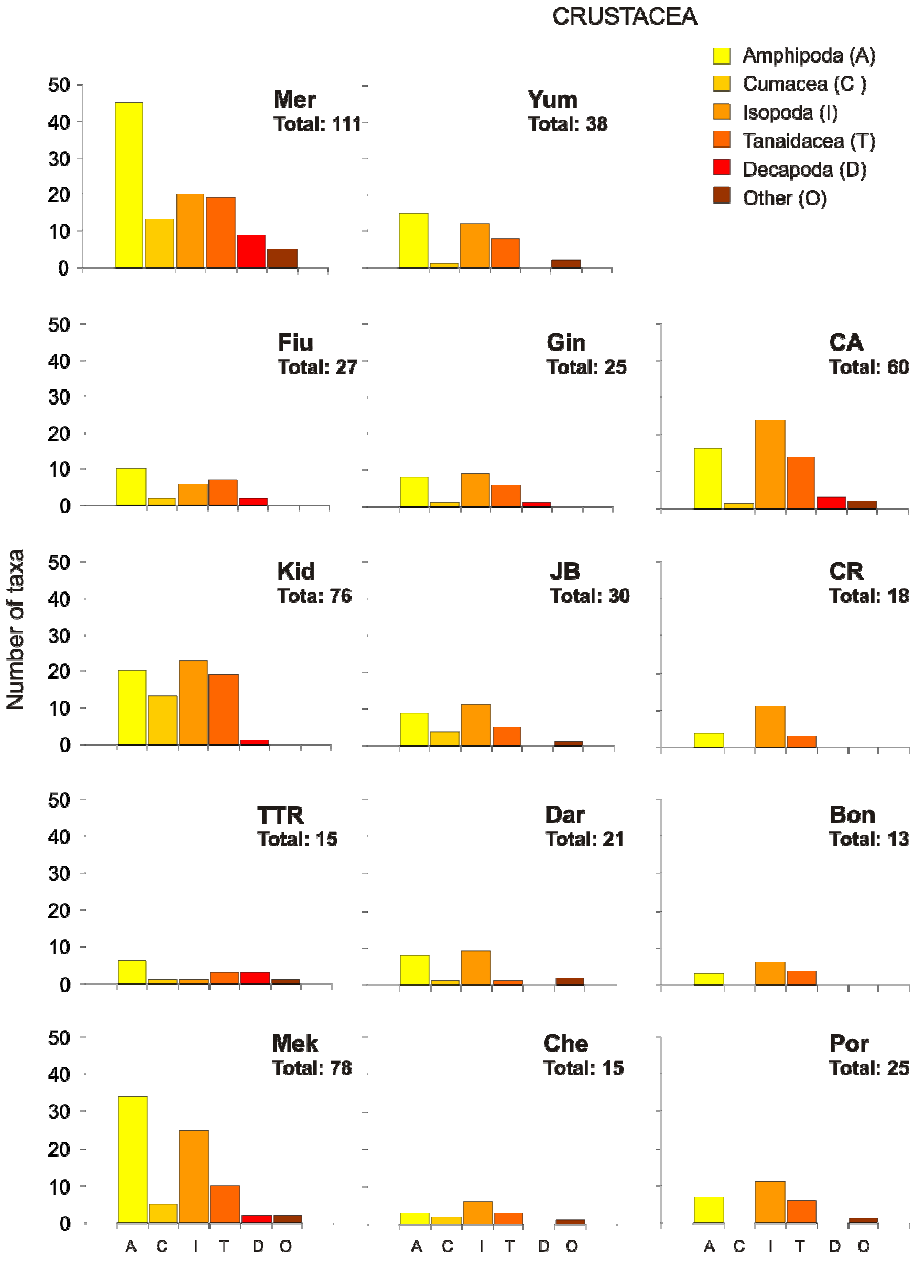

Figure 2.1.9. Taxa richness of different groups of Crustacea in the different mud volcanoes studied. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). The shallow Moroccan field includes Mercator (Mer), Fiúza (Fiu) and Kidd (Kid); the Western Moroccan field includes TTR, Meknès (Mek), Yuma (Yum), Ginsburg (Gin), Jesuz Baraza (JB) Darwin (Dar), Chechouan (Che) and the deep-water field includes Captain Aruryunov (CA) Carlos Ribeiro (CR), Bonjardim (Bon) and Porto (Por).

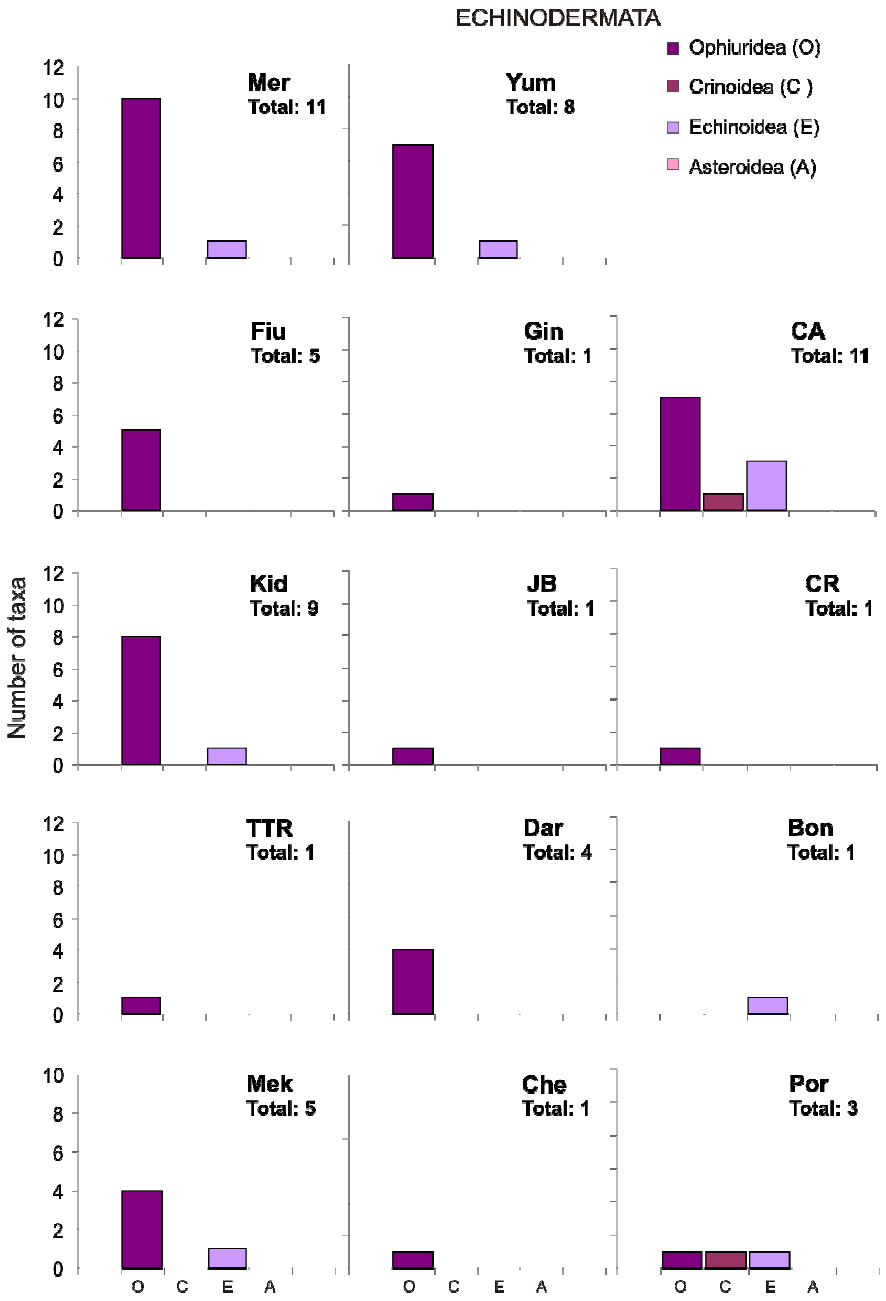

Figure 2.1.10. Taxa richness of different groups of Echinodermata in the different mud volcanoes studied. The values are pooled from all samples in each site. Note that the number of samples and sampling gear vary among sites (see Annex I). The shallow Moroccan field includes Mercator (Mer), Fiúza (Fiu) and Kidd (Kid); the Western Moroccan field includes TTR, Meknès (Mek), Yuma (Yum), Ginsburg (Gin), Jesuz Baraza (JB) Darwin (Dar), Chechouan (Che) and the deep-water field includes Captain Aruryunov (CA) Carlos Ribeiro (CR), Bonjardim (Bon) and Porto (Por).

The isopod family Desmossomatidae is the most frequent occurring in 13 of the 17 of the mud volcanoes studied. The amphipod families Phoxocephalidae and Caprellidae occur in 12 mud volcanoes, the tanaid family Apseudidae and the isopod family Munnopsidae in 11 mud volcanoes and the cumacean family Nannasticidae in 10 mud volcanoes. The most frequent decapod family, the Xanthidae occur only in 5 mud volcanoes.

Echinodermata. The echinoderms were a much less common group in the mud volcano samples; the 481 individuals were ascribed to 20 species from which 13 were ophiuroids (Figure 2.1.10). The ophiuroids were recorded from the shallowest (Al Idrisi, 230m) to the deepest (Porto, 3862m) mud volcano and their distribution is given in detail in Chapter 2.5).

2.1.3.3.Chemosynthetic species

The studies on the macrobenthic assemblages revealed a remarkable high diversity of chemosymbiotic species in the Gulf of Cadiz many of which are new to science (Hilário *et al*. 2008, Hilário *et al*. submitted; this thesis Chapter 2.2: Rodrigues *et al*. 2008; and Chapter 2.3: Oliver *et al*. in prep.). Until now 30 chemosymbiotic species, ten bivalves and twenty frenulate siboglinids (A. Hilário pers. comm.) were identified as inhabiting the different mud volcanoes in the Gulf of Cadiz (Table 2.1.3). Two other species *Laubericoncha chuni* and *Pliocardia* sp. were also found in Bonjardim mud volcano but the occurrence of living specimens could not be confirmed as only empty separate valves were collected.

In most cases the trophic status of these species has been confirmed either by stable isotope analysis (Chapter 3.1) or endosymbiont DGGE analysis of bacterial 16S rRNA genes and clone libraries (Chapters 3.2: Rodrigues *et al*. submitted and 3.3).

Overall there is a trend of increased diversity of chemosymbiotic species with increased depth; the shallower (<1000m) mud volcanoes yielded 1-5 species (Mercator: 4 species; Darwin: 5 species) while the deeper ones may yield up to nine species (Captain Arutyunov: 8 species; Bonjardim: 9 species). The distribution of chemosymbiotic bivalves is discussed in more detail in Chapter 2.3.

Table 2.1.3. List of chemosynthetic species found at different mud volcanoes. Question mark indicates possible but not yet confirmed chemosynthesis. F: Fiuza, Mr: Mercator, Ge: Gemini; K: Kidd, Mk: Meknès; Y: Yuma, G: Ginsburg, JB: Jesuz Baraza, D: Darwin, CA: Captain Arutyunov, Sa: Sagres; Se: Semenovich; CR: Carlos Ribeiro, B: Bonjardim, P: Porto. Data on Frenulata from Hilário *et al*. submitted.

	F	Mr	Ge	K	Mk	Y	Gi	JB	D	CA	Sa	Se	CR	B	P
Frenulata															
Siboglinum sp1						X	X		X						
Siboglinum sp2		X	X	X											
Siboglinum sp3															
Siboglinum sp4		X								X					
Siboglinum sp5									X						
Siboglinum sp6					X										
Siboglinum cf *poseidoni*										X					
Polybrachia sp1														X	
Polybrachia sp2												X	X		
Polybrachia sp3											X				
Lamelisabella sp.														X	
Lamelisabella denticulata															X
Spirobrachia tripeira															X
Bobmarleya gadensis															X
Bivalvia															
Acharax sp. nov						X	X	X		X			X		X
Petrasma sp. nov	X	X		X	X	X	X		X						
Lucinoma sp. nov		X													
Thyasira vulcolutre										X			X		
Spinaxinus cf *sentosus (?)*										X					
Calyptogena regab														X	
Isorropodon sp. nov (?)										X					
Isorropdon cf. *perplexum*										X					
Bathymodiolus mauritanicus									X						
Idas sp													X		

2.1.3.4.Quantitative results – density and diversity indexes

Quantitative data were compiled from processed samples taken during TTR (6 USNEL boxcores) and MSM01-03 (10 USNEL boxcores and several multicores). The cumulative species vs area curves were plotted for six mud volcanoes (Figure 2.1.11). The total area sampled in each mud volcano was less than $1m^2$ except for Mercator ($1.4m^2$). In all cases the cumulative curve shows steep increases in the number of species for every new sample added indicating that, even for the best sampled mud volcano, the area sampled is clearly insufficient to fully represent the species richness in these habitats. We can therefore expect that the collection of more samples will continue to add more records to the current species list of each mud volcano.

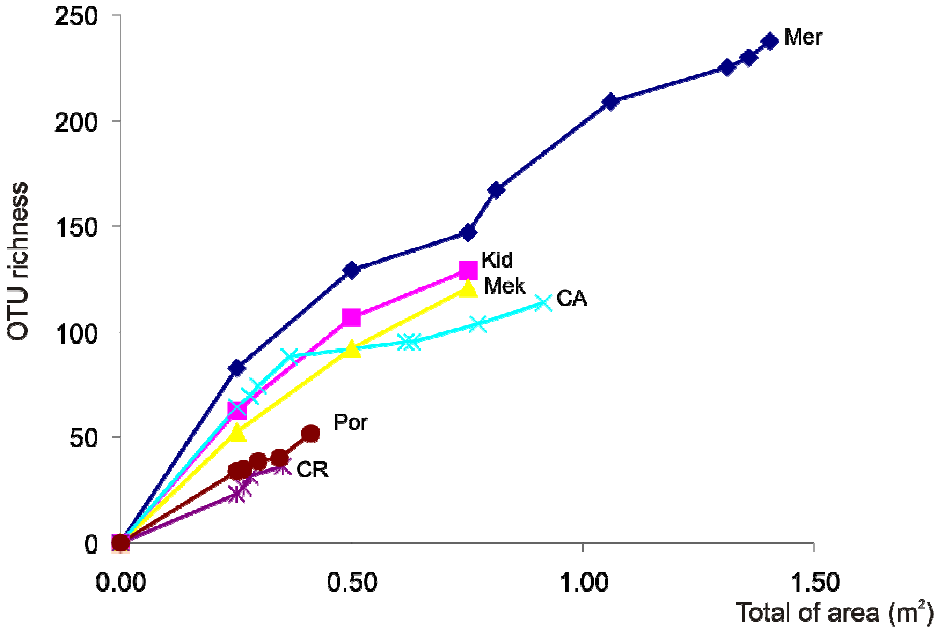

Figure 2.1.11. Species accumulation versus total sampled area in different mud volcanoes from the Gulf of Cadiz. Mer: Mercator; Kid: Kidd; Mek: Meknès; CA: Captain Arutyunov; CR: Carlos Ribeiro; Por: Porto.

The provisional estimates of taxa richness, density and composition of the benthic assemblages showed important variability among mud volcanoes but also within each mud volcano (Figure 2.1.12 and Table 2.1.4). Taxa richness per sample varied from 47 to 107 in the shallower mud volcanoes and was consistently lower in the deeper mud volcanoes (23-34). The exception was Captain Arutyunov where the two crater samples showed contrasting values (21 and 64 taxa). The same trends were apparent from H' and $Es_{(n)}$ values although equitability maintained rather high values in most samples (Table 2.1.4). Concerning density, there was a higher variability among and within mud volcanoes but the general depth-related trend was retained: values of approx. 200-600 ind.m^{-2} in the deeper mud volcanoes and 600-2700 ind.m^{-2} in the shallower mud volcanoes with extreme values in Captain Arutyunov (up to 3000 ind.m^{-2}) .It should be noted that siboglinid densities were not accounted for these estimates.

In mud volcanoes where samples from different locations (crater, flanks and off-mound) were available different patterns were observed: In Mercator diversity (S, H', $ES_{(n)}$) and density were higher in the crater decreasing in the

flanks and further off-mound while in Kidd and Meknès values were rather constant with slightly higher diversity values (H' and $Es_{(n)}$) off-mound and slightly higher densities in the flanks of Kidd. Because of the insufficient number of replicates these observations are inconclusive.

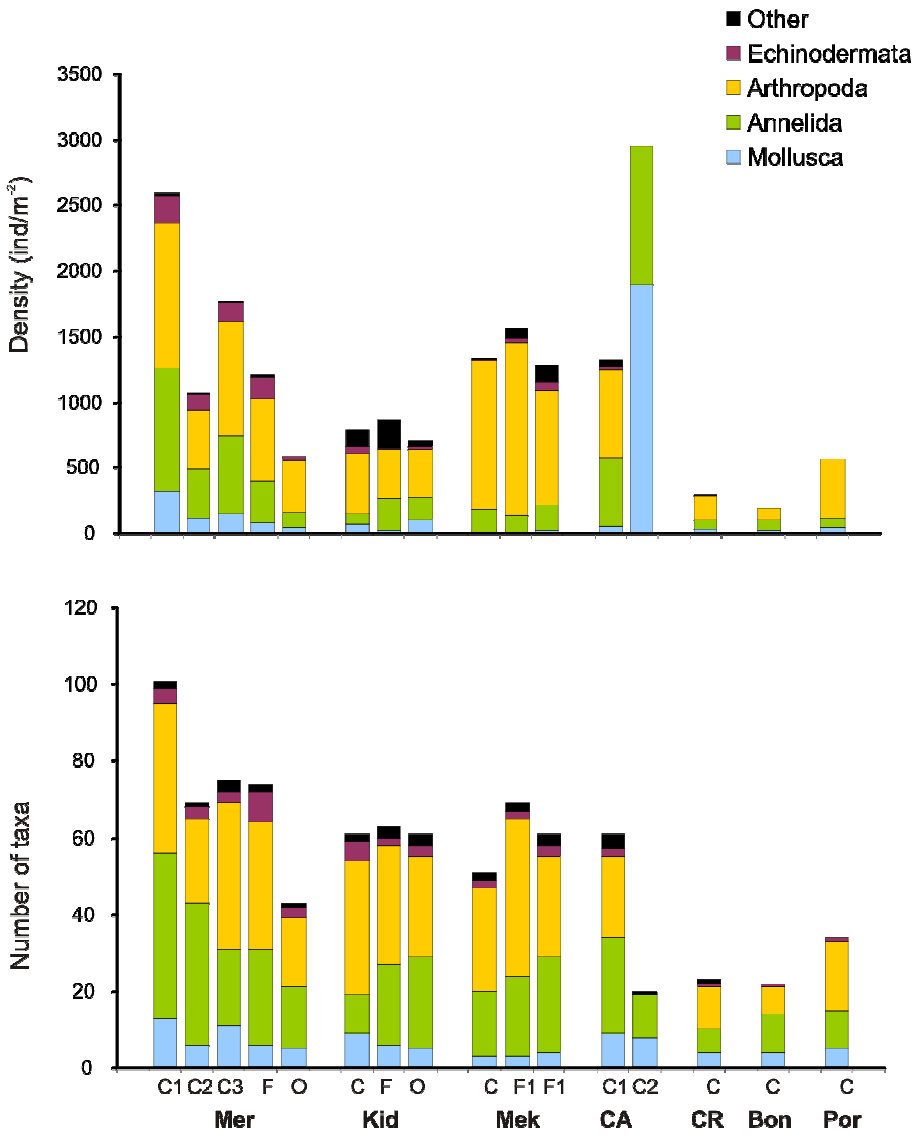

Figure 2.1.12. Macrofaunal taxa richness and density in individual samples (USNEL boxcore; Area: 0.25m^2) from seven different mud volcanoes in the Gulf of Cadiz. C: crater, F: Flank and O: off-mound. Numbers correspond to different stations numbers (Table 2.1.4). Mer: Mercator; Kid: Kidd; Mek: Meknès; CA: Captain Arutyunov; CR: Carlos Ribeiro; Por: Porto.

Table 2.1.4. Univariate biodiversity indices for the 16 USNEL sampled. S: number of species,N: number of individuals per replicate; H'(ln): Shannon-Wiener diversity index; J': Pielou's eveness index; $ES_{(n)}$ Hulbert´s expected species richness. CA: Captain Arutyunov; CR: Carlos Ribeiro; C: crater sites; F: flank sites; O: off mud volcano sites.

	Cruise	Station	S	N	H'	J'	$ES_{(50)}$	$ES_{(100)}$
Mercator	TTR	575 (C3)	83	499	3.548	0.803	26.0	38.7
		576 (F)	78	316	3.740	0.858	29.1	43.9
		577 (O)	43	147	2.957	0.786	23.0	---
	MSM01.03	241 (C1)	107	680	4.003	0.857	31.1	46.7
		242 (C2)	75	325	3.621	0.839	27.7	41.8
Kidd	TTR	559 (O)	63	181	3.739	0.903	30.7	46.5
		560 (C)	62	198	3.627	0.878	28.8	43.5
		561 (F)	63	216	3.620	0.874	28.4	42.4
Meknès	MSM01.03	319 (C)	53	365	3.099	0.780	21.2	30.0
		321 (F1)	73	423	3.585	0.836	26.5	38.6
		335 (F2)	65	327	3.519	0.843	26.0	38.1
CA	MSM01.03	180 (C1)	64	358	3.243	0.780	22.6	33.6
		218 (C2)	21	740	1.760	0.578	8.5	10.5
CR	MSM01.03	157 (C)	23	73	2.684	0.856	18.9	---
Bonjardim	MSM01.03	133 (C)	23	53	2.742	0.874	22.2	---
Porto	MSM01.03	145 (C)	34	143	2.937	0.833	20.6	29.0

The macrofaunal assemblages showed high evenness at species level (Table 2.1.4) but were usually dominated by crustaceans and polychaetes (Figure 2.1.12). Again, please note that siboglinids were not accounted for. In some cases they show high density aggregations that may substantially change these provisional results. Amphipods and isopods followed by polychaetes accounted for approximately 80% of the total abundance in Mercator and Kidd (shallow Moroccan field); ophiuroids were also well represented in Mercator (ca. 10% of total abundance). Crustaceans were clearly dominant (70-90% of total abundance) in Meknès (Western Moroccan field) where polychaetes always accounted for less than 15% of total abundance and molluscs had very low abundance. The deeper mud volcanoes showed low abundance and very heterogeneous assemblages: isopods, tanaids and polychaetes shared 80% of the total abundance in Bonjardim and Porto but in the latter crustacean abundance was further reinforced by the contribution of amphipods. In Carlos Ribeiro isopods accounted for 50% of total abundance followed by amphipods and polychaetes. Once again Captain Arutyunov stood out by showing in one of the samples a completely different

assemblage. This sample (MSM01.03 180, marked as C1 in Figure 2.1.12) was collected from a very active area of the mud volcano and the sediments recovered contained a large amount of gas hydrates. The assemblage showed the lowest evenness values (J':0.578; Table 2.1.4) as it was it was dominated by high numbers of an ampharetid polychaete, small chemosymbiotic bivalves (*Thyasira vulcolutre* and *Isorropodon* sp. nov.) and minute gastropods (unknown species). No siboglinids were collected in this particular sample.

Differences in the biodiversity of the seven mud volcanoes are illustrated by rarefaction curves plotted in Figure 2.1.13. Rarefaction curves represent the expected number of species present at any random selection of samples within a location and have been recommended over traditional accumulation curves to avoid distortions in the curves due to variations in species abundance and sampling effort (Gotelli & Colwell 2001). The curves drawn for Porto, Bonjardim and Carlos Ribeiro are based in a very low number of individuals (less than 150) but nevertheless there is a clear pattern of decreasing biodiversity with increasing depth of the mud volcanoes: Mercator and Kidd (shallow Moroccan field) show very similar curves with the highest expected biodiversity (steepest slope), Captain Arutyunov, Carlos Ribeiro, Bonjardim and Porto (deep-water field) are grouped together with the lowest expected biodiversity and Meknès (Western Moroccan field) has intermediate values.

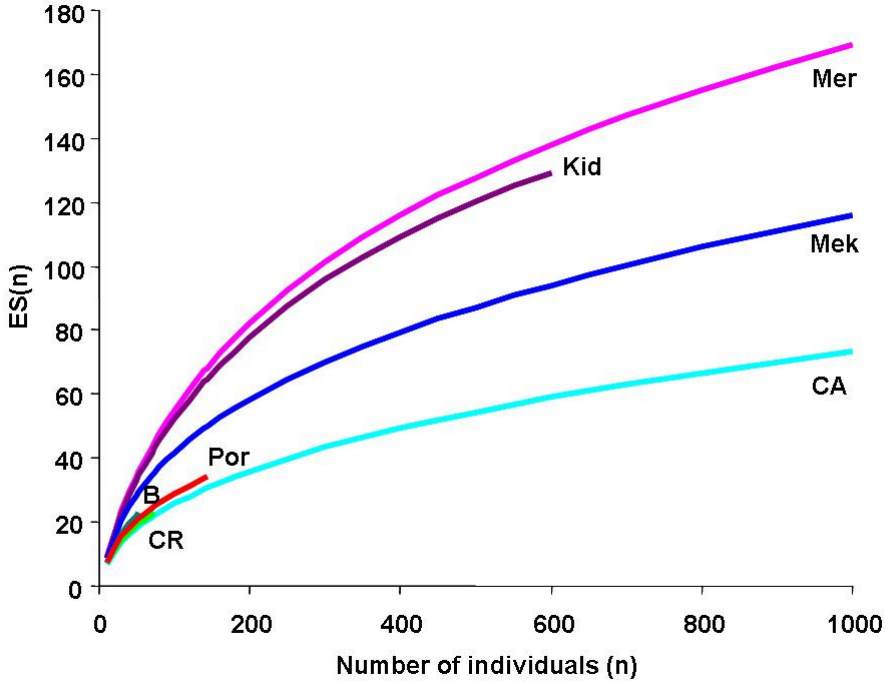

Figure 2.1.13. Species rarefaction curves in different mud volcanoes from the Gulf of Cadiz. Mer: Mercator; Kid: Kidd; Mek: Meknès; CA: Captain Arutyunov; CR: Carlos Ribeiro; B: Bonjardim; Por: Porto.

2.1.3.5.Regional biodiversity and species turnover

The complete dataset of taxa collected in 64 samples from the 17 mud volcanoes was used to examine the rates of species addition, loss and turnover along the depth gradient (Figure 2.1.14). As described above there is a high variability in the species richness and composition of the different mud volcanoes and the abrupt discontinuities in the regional species accumulation curve reflect this faunal turnover at each mud volcano or group of mud volcanoes.

The shallow Moroccan field (Al Idrisi, Mercator, Fiuza, Kidd and TTR) shows the highest biodiversity and accumulates 62% of the recorded taxa, the Western Moroccan field (Meknès, Student, Yuma, Ginsburg, Darwin, Jesuz Baraza, Aveiro and Chechouen) adds more 20% of the species, Captain Arutyunov alone adds another 11% and the remaining 7% of records are contributed by the deeper mud volcanoes (Carlos Ribeiro, Bonjardim, Porto. The highest turnover occurs when samples from the Captain Arutyunov are

added. Although this mud volcano contributes with a significant proportion of added records the high turnover is also due to the high number of species lost (species with shallower distributions that are never found at greater depths).

The two major areas of enhanced turnover, marked by the Mercator and Captain Arutyunov at 350 and 1300m respectively coincide with important oceanographic features (Figure 2.1.14). Mercator is located approximately at shelf break depth, at the boundary of the surficial Atlantic water and the Mediterranean Outflow Water while Captain Arutyunov rises at the boundary of the Mediterranean outflow Water and the North Atlantic Deep water.

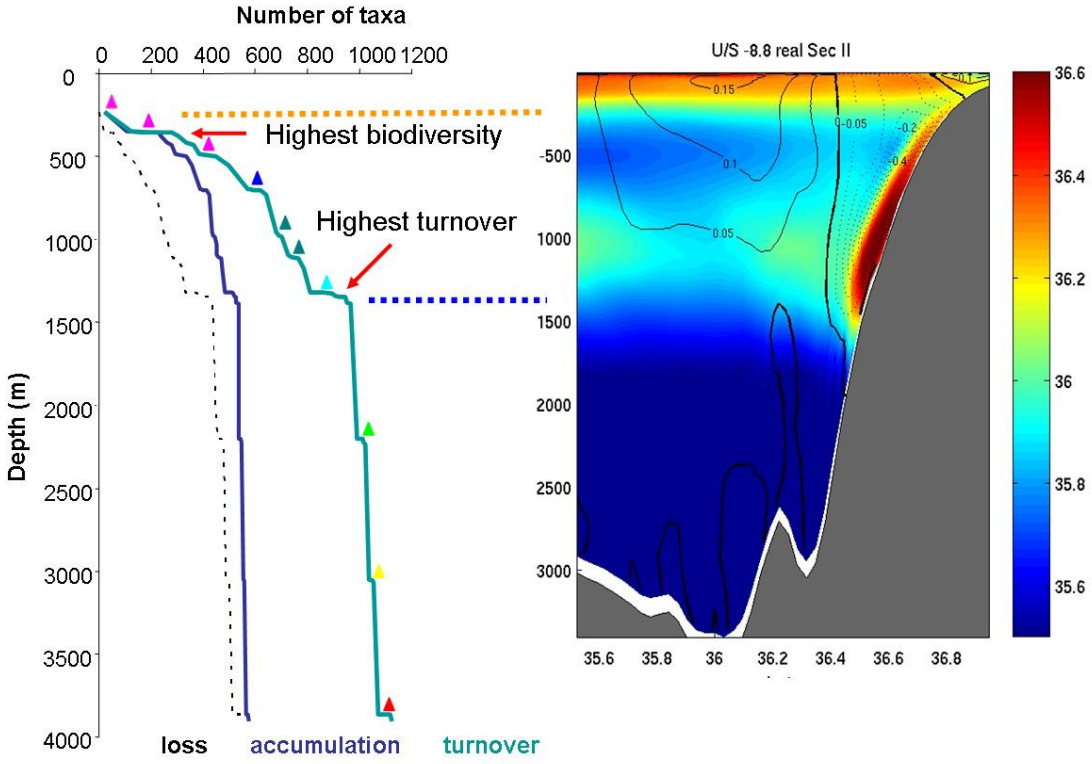

Figure 2.1.14. Regional species accumulation, loss and turnover curves along the depth gradient in the Gulf of Cadiz. The triangles mark the steps in the turnover curve associated with different mud volcanoes. The figure in the right-hand (from Peliz *et al*. 2006) shows the salinity signature of different water masses in a selected vertical section of the Gulf of Cadiz. The depth scales of both figures are matched in order to show the coincidence of major features in the turnover curve with major changes in water mass characteristics: the surface layer of Eastern North Atlantic Central Water, the Mediterranean Water with the upper and lower cores located between 500 and 1200m and the North Atlantic Deep Water flowing at greater depths.

2.1.4.Discussion

One of the major gaps in the present knowledge of cold seep assemblages is the low taxonomic resolution of most published studies particularly in what concerns the accompanying macrofauna. The main emphasis of cold seep research is on the typical symbiont-bearing taxa and among the few exceptions are the studies focused on other groups such as the Anthozoa (López-González *et al*. 2003), the Decapoda (Martin & Haney 2005) or the Ophiuroidea (Stöhr & Segonzac 2005).

Background macrofaunal assemblages. Usually, when non-typical taxa are mentioned, the identifications are given at the family, order or even class level (see review by Levin 2005). Gastropod aggregations (Archaeogastropoda and Neogastropoda) frequently found in hydrothermal vent assemblages (Galkin 1997) and were also reported as common in microbial mats at Hydrate Ridge (Sahling *et al.* 2002). In the Gulf of Cadiz similar aggregations occur in Captain Arutyunov and to a lesser degree also in Darwin. These gastropods are seemingly bacterial grazers and although large bacterial mats were not detected in these two mud volcanoes the preliminary results from measurements taken during the MSMerian (in Captain Arutyunov) and JC10 cruises (in Darwin) point out to a relatively high methane emission and subsequently a high bacterial activity in these mud volcanoes (Pfannkuche 2007; Weaver & Masson 2008).

An overwhelming dominance of the Canalipalpata polychaete family Ampharetidae (*Amphisamytha* sp.) was reported from Florida seeps and Guaymas Basin hydrothermal sediments (Levin & Mendoza 2007), Cascadia Margin (Sahling *et al*. 2002) and at Barbados Prism, in this case together with other Canalipalpata (Chaetopteridae) and Scolecida (Maldanidae) families (Olu *et al.* 1996, 1997). The Aciculata families Polynoidae and Dorvillidae were abundant at Cascadia Margin (Sahling *et al*. 2002) and the Glyceridade, *Glycera tesselata*, was also reported from the Gulf of Mexico (Bergquist *et al*. 2003). Polychaetes are frequently reported as the dominant background fauna in cold seeps and the Gulf of Cadiz is not an exception only rivaling in number of species and abundance with the peracarid crustaceans. The three major groups of Polychaetes were all well represented. The Canalipalpata are

predominantly small sized, low mobility, surface and subsurface deposit feeders (Fauchald & Jumars 1979; Rouse & Pleijel 2001) and they may occupy the mud volcanoes in a rather oportunistic way taking profit of the high organic content of the sediments and reaching high abundances. They are represented in the Gulf of Cadiz by families such as the Ampharetidae and the Fauveliopsidae with their lowest contribution in the mud volcanoes from the Western Moroccan margin where the widespread occurrence of authigenic carbonates often seals the seafloor surface by cementic the soft sediments. The Aciculata are frequently large-sized, mobile carnivores and omnivores (Fauchald & Jumars 1979; Rouse & Pleijel 2001). Very frequent but not as abundant as the Canalipalpata families, the Glyceridae, Polynoidae and also Onuphidae in the Gulf of Cadiz are usually members of the background community that prey upon the small crustaceans and other polychaetes that are very abundant in cold seep sediments. The contribution of chemosynthetic production for the nutrition in some of these polychaete species (probably via bacterial grazing, omnivory or carnivory) has been confirmed by the depleted $\delta^{13}C$ signature of their tissues (Chapter 3.1).

To date, more than 125 species representing 33 families of decapods have been reported on hydrothermal vents and cold seeps (Martin & Haney 2005). The thalassinoid ("mud shrimps") families Axiidae, Callianassidae and Calocaridae were reported from the Gulf of Mexico (Martin & Haney 2005). Within the Anomura, species of the family Galatheidae (squat lobsters) most belonging to the genera *Munida* and *Munidopsis*, are frequently reported from reduced environments. Although some galatheid species are known only from these environments, they are not probably endemics but rather vagrants as most of the other decapod families (Martin & Haney 2005). In the Gulf of Cadiz the Callianassidae are represented by a new species*, Vulcanocalliax arutyunovi,* collected from Captain Arutyunov (Dworschak & Cunha 2007) and the Galatheidae are represented by the frequent *Munida* sp., especially in the shallower Morocan margin, and by *Munidopsis acutispina* recorded in the deepest mud volcano, Porto. Ten other decapod families occur in the Gulf of Cadiz from which two (Alpheidae and Cymonomidae) were not previously recorded in seeps. The most frequent species was a common Xanthidae in the

area, *Monodaeus couchi*. The $\delta^{13}C$ and $\delta^{15}N$ signatures of its tissues (Chapter 3.1) are indicative of a possible predatoty and or scavenging activity on smaller animals living in the mud volcano sediments.

Despite the high contribution of peracarid crustaceans in number of species and abundance for the macrofaunal assemblages of the Gulf of Cadiz the mostly lacking records from other cold seep regions impairs any comparisons. Biogenic microhabitat heterogeneity is a major driver for the diversity of peracarid crustaceans but other factors include sedimentological hydrological conditions, disturbance created by feeding and burrowing activities or other macrofaunal animals, predation and availability of food resources (reviewed by Brandt 1997). These may explain why crustacean peracarids are most well represented in the shallower mud volcanoes where where a wide range of microhabitats occur, food resources are more diverse and the biogeochemical conditions typical of cold seeps are generally milder enabling a greater penetration of the background fauna.

Echinoderms are not frequently reported from deep-sea reducing environments. Nevertheless, ophiuroids presented an unusual vast distribution in the mud volcanoes from the Gulf of Cadiz analysed in detail in Chapter 2.5.

Chemosymbiotic species. In the shallower mud volcanoes of the Gulf of Cadiz the chemosynthetic species, mostly solemyid and lucinid bivalves and *Siboglinum* spp. live buried inside the sediments, a distribution that is probably related to the deep position of the sulphide/methane gradient, usually located at more than 30 cm below the surface (e.g. Van Rensbergen *et al*. 2005). The schemes of differing chemosymbiotic assemblages related with the location of the sulphide/methane gradient and the intensity of fluxes proposed by several authors (Sahling *et al*. 2002; Treude *et al*. 2003) are consistent with the distribution of different chemosymbiotic taxa and the inferred biogeochemical conditionsof the sediments infererred from the presently available data in the Gulf of Cadiz. In the deeper mud volcanoes dense aggregations of living chemosynthetic species were found: small vesicomyids live very close to the surface in the Captain Arutyunov in areas where a large amount of gas hydrates occur a few centimeters below the surface while in more peripheric areas large densities of thin siboglinids were

recorded (Pfannkuche 2006). In Carlos Ribeiro where the mud breccia is stiffer and the gas hydrates occur more sparse and deep in the sediments, the very central area is occupied by thyasirids and the long frenulate *Bobmarleya gadensis* occupies a more peripheric zone (MR Cunha pers. observ. during JC10 cruise) while in Porto many clumps of the large frenulates *Spirobrachia tripeira* and *Lamellisabella denticulata* are widespread over all the crater (Akhmetzhanov *et al*. 2006; Pfannkuche 2006).

Because frenulates absorb reduced compounds through the posterior parts of their tubes, the oxidation-reduction (redox) profile of the sediment is expected to play a role in determining which species are able to inhabit a particular site (Dando *et al*. 2008). Shorter species are expected to live in mud volcanoes with higher concentrations of reduced compounds in upper layers of the sediment and vice-versa. Large frenulates with long bodies are expected to bridge wider redox boundaries, living in sediments where reduced compounds are expelled in seep fluids or diffused to the upper sediment layers (Hilário *et al*. submitted). These hypotheses might help to explain the presence of the long-bodied (> 30 cm) *Lamellisabella*, *Spirobrachia* and *Bobmarleya* in Porto, Bonjardim and Carlos Ribeiro mud volcanoes where high sulphide concentrations are 50 cm below the sediment interface (Hensen *et al*. 2007; Nuzzo *et al*. 2008).

The number of chemosynthetic species in the Gulf of Cadiz is remarkably high when compared to the other cold seep regions in the Atlantic and Mediterranean (Table 2.1.5). Contrasting to the 30 species found in the Gulf of Cadiz, the other cold seeps present only two to six chemosymbiotic species.

Among the Frenulata *Sclerinium contortum* and *Oligobrachia haakonmosbiensis* are restricted to the crater zone of the Haakon Mosby mud volcano (HMMV) (Pimenov *et al.* 2000; Gebruk *et al*. 2003); *S. contortum* also occurs at the Storegga Slide and the Nyegga; *Lamellibrachia* sp. nov. and undetermined species of *Siboglinum* have been reported on several mud volcanoes from the eastern Mediterranean (Napoli, Milano, Maidstone, Moscow, Anaximander, Amsterdam, Kazan and Kula) (Olu-Le Roy *et al.* 2004) and the Vestimentifera *Escarpia southwardae* was found at cold seeps off the western coast of Africa in Congo and Angola Basins (Olu-Le Roy *et al*. 2007);

Siboglinum poseidoni and *Lamelisabella denticulata* have been found in methane seeps in the Skagerrak and in the Gulf of Biscay, respectively (Dando *et al*. 1994; Hilário & Cunha 2008). Among the bivalves, five species occur in the Eastern Mediterranean, three in the Southeast Atlantic and only one in the Norwegian margin: small Thyasiridae bivalves mainly have been associated with Siboglinidae fields at the HMMV (Pimenov *et al*. 2000; Gebruk *et al*. 2003); *Idas modiolaeformis*, *Myrtea amorpha*, *Lucinoma kazani*, *Isorropodon perplexum* and *Thyasira striata* have been reported from the eastern Mediterranean mud volcanoes (Olu-Le Roy *et al*. 2004); and bivalves of the families Mytilidae (*Bathymodiolus* sp.) or Vesicomyidae (*Calyptogena regab*, *Laubericoncha chuni*) dominates the faunal assemblages of the western coast of Africa (Congo and Angola Basin) (Olu-Le Roy *et al*. 2007). A more detailed discussion on the distribution of chemosymbiotic bivalves is provided in Chapter 2.3.

Most species are known only from one region, only a few are common to two regions and none occurs in three regions. The Gulf of Cadiz shares the frenulate species *Siboglinum poseidoni* and *Lamelisabella denticulata* with reducing environments in the Skagerrak and the Gulf of Biscay, respectively. Among the Bivalves *Bathymodiolus mauritanicus*, and *Calyptogena regab* are found both in the Gulf of Cadiz and in the South East Atlantic off Africa. *Lauberioconcha chuni* known from the Angola Basin were recently found also in the Gulf of Cadiz (Bonjardim) but only as empty shells.

Biodiversity and distributional patterns. One of the achievements of the work carried out in the Gulf of Cadiz is the continued effort to produce a comprehensive list with a higher taxonomic resolution. In fact, the 507 taxa identified to species or genus level from a total of over 700 in the Gulf of Cadiz constitute a rare exception in cold seep studies and together with the high number of new species detected testify the great biodiversity interest of the region.

Table 2.1.5. Comparision among European cold seeps of the presence of chemosymbiotic species. HMMV: Haakon Mosby Mud Volcano, StS: Storegga Slide; GoC: Gulf of Cadiz, MedR: Mediterranean Ridge, AM: Anaximander Mounts, NDSP: Nile deep sea fan, GoG: Gulf of Guinea, AngB: Angola Basin.

	Norwegian margin		**GoC**	**Eastern Mediterranean**			**South East Atlantic**	
Chemosymbiotic species	HMMV	StS		MedR	AM	NDPF	GoG	AngB
Annelida								
Vestimentifera								
Escarpia southwardea							X	X
Lamellibrachia new species				X				
Frenulata								
Bobmarleya gadensis			X					
Lamelisabella denticulata			X					
Oligobrachia sp.			X					
Oligobrachia haakonmosbiensis	X	X						
Polybrachia spp.			X					
Siboglinum spp			X	X				
Siboglinum poseidoni			X					
Spirobrachia sp.			X					
Spirobrachia tripeira			X					
Monilifera								
Sclerolinum contornum	X	X						
Bivalvia								
Solemyidae								
Acharax sp.			X					
Petrasma sp.			X					
Lucinidae								
Lucinoma sp.			X					
Lucinoma kazani				X	X	X		
Thyasiridae			X					
Thyasira sp.	X	X		X	X			
Thyasira vulcolutre			X					
Mytilidae								
Bathymodiolus sp.								X
Bathymodiolus mauritanicus			X					
Idas sp.			X			X		
Idas modiolaeformis				X				
Vesycomidae		X						
Calyptogena regab.			X					X
Laubericoncha chuni			X				X	X
Isorropodon new species			X					
Isorropodon perplexum			X	X	X			

As discussed above the diversity of chemosymbiotic taxa in the Gulf of Cadiz is remarkably higher than in other known seepage regions. Besides the many new species of typical seep fauna also among non-seep fauna new species and cryptic diversity were revealed (Annex II; Moura *et al.* 2008). Because of the general poor knowledge of species distributions in deep-sea ecosystems the endemic status of many of the species discovered in the Gulf of Cadiz remains to be proved. The speciation is dependent of the relation

between mode of reproduction, number of eggs produced, distribution patterns, zoogeography, physiological tolerance and genetic variability among other factors (Sanders 1977). The Gulf of Cadiz privileged location as a biogeographic crossroad with the oceanographic circulation establishing pathways for organism dispersal and the high environmental heterogeneity may be hypothesised to favour speciation and to sustain the high biodiversity of the region.

The macrofaunal assemblages of the mud volcanoes in the Gulf of Cadiz display a wide variability in species composition and structure. The enhanced faunal turnover and sharp change in the structure and composition of the benthic assemblages at approximately 1300m depths clearly matches the changes in oceanographic conditions but it is also well known that the deeper mud volcanoes are generally more active with gas hydrates and higher fluxes of methane frequently observed. The benthic assemblages from the mud volcanoes in the shallow and western Moroccan fields show high biodiversity and density, mainly of background non-seep fauna with low degree of endemicity. These features are more pronounced in the shallow Moroccan field where the proximity to the coast as well to the euphotic layer greatly enhances the availability of different food sources and the productivity of the area. The typical seep fauna is represented by a lower number of inconspicuous species such as the small-sized *Siboglinum* spp. and buried bivalves (Solemyidae and Lucinidae). In the deeper mud volcanoes species richness and density of the assemblages are generally much lower but the degree of endemicity is clearly higher and mud volcanoes are colonized by a high diversity of typical seep fauna. Large-sized Siboglinidae sometimes forming conspicuous clumps and fields of Thyasiridae or Vesicomyidae bivalves may reach high densities in patchy aggregations.

Densities from the different mud volcanoes commonly varied from a few hundred to thousands per square meter. Values reported for other seeps in the Atlantic (Olu *et al*. 1997; Levin *et al.* 2000; Levin & Mendoza 2007) are usually higher but the values estimated for the Gulf of Cadiz do not account for siboglinid densities which can occur in local patches with more than 20,000 ind.m^{-2} (MR Cunha pers. comm.). In some mud volcanoes samples from

different locations (crater, flanks and off-mound) were available but no consistent patterns of zonation in diversity and density were observed. Moreover, because of the insufficient number of replicates these observations are inconclusive. The comparisons of seep versus non-seep densities are not consistent in the different regions studied (reviewed by by Levin 2005) and in fact several studies reported similar densities of seep and background assemblage (e.g. Sagami Bay, at 1170m: Shirayama & Ohta 1990; Northern California slope at 500m: Levin *et al.* 2003).

Although general trends in density and biodiversity can be recognized and identified with the main bathymetric fields of mud volcanoes it is also true that each mud volcano yields a particular and distinctive faunal assemblage. Factors affecting the biodiversity and species distributional patterns in seeps are likely to include porewater hydrogen sulphide concentrations and fluxes, oxygen availability, sediment structural characteristics (Sahling *et al*. 2002; Levin *et al*. 2003) but also biologically generated habitat heterogeneity (Bergquist *et al*. 2003). The mechanisms by which biogenic structures give rise to these patterns include provision of food resources, living space, favorable settlement conditions and refuge from predation and environmental stress (reviewed by Bergquist *et al*. 2003). At a smaller scale, the patchy structure of the community is also the result of local effects creating habitat heterogeneity such as the feeding and reproductive activities of burrowing and scavenging invertebrates that form mounds and depressions on the sediment surface. The benthic macrofaunal taxa are dependent for their existence upon events occurring at the sediment surface. They require contact with the surface, either constantly or periodically, directly or indirectly through biogenic structures and sediment reworking (Cosson *et al.* 1997).

The relationship between natural drivers and the biodiversity and distributional patterns of mud volcano biological assemblages is still poorly understood owing to the generalized lack of integration between biological and environmental sampling. The oceanographic setting and wide bathymetric range of the known seep sites, the variability in biogeochemical conditions within and between mud vocanoes and the biologically generated habitat

heterogeneity are certainly major drivers for the biodiversity and functioning of the mud volcano benthic assemblages that await further investigations.

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Chapter 2.2.Thyasiroidea (Mollusca: Bivalvia) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic)

Abstract

The Thyasiroidea collected from the mud volcanoes of the Gulf of Cadiz are reviewed. Of the seven species identified only one, *Thyasira vulcolutre* n. sp., is closely associated with a chemosynthetic setting. This species has anatomical features typical of chemosymbiotic taxa and is compared with *T. sarsi* (Philippi, 1845)*, T. southwardae* Oliver & Holmes, 2006, *T. oleophila* Clarke, 1989 and *T. methanophila* Oliver & Sellanes, 2005. The other six, *Thyasira (Parathyasira) granulosa* (Monterosato, 1874), *Thyasira tortuosa* (Jeffreys, 1881), *Thyasira obsoleta* (Verrill & Bush, 1898), *Axinulus croulinensis* (Jeffreys, 1847), *Mendicula ferruginosa* (Forbes, 1844) and *Leptaxinus minutus* Verrill & Bush 1898, are previously known from typical deep-water benthic settings. Of these only *A. croulinensis* is known to harbour chemosymbionts although the gill anatomy of *T. tortuosa* suggests that it might also be chemosymbiotic. *Thyasira vulcolutre* is restricted to active seeps but there is no pattern in the distribution of the other species.

Key words: *Thyasira*, new species, bathyal, seep

Rodrigues CF, Oliver PG & Cunha MR (2008) Thyasiroidea (Mollusca: Bivalvia) from the mud volcanoes of the Gulf of Cadiz. *Zootaxa*, **1752: 41–56**.

Thyasiroidea (Mollusca: Bivalvia) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic)

2.2.1.Introduction

Communities of benthic animals associated with cold seeps are known from many locations on active and passive continental margins around the world and occur at virtually all depths (Sibuet & Olu 1998; Levin 2005). Along the European margins cold seepage is known from the North Sea and Norwegian margin (e.g. Dando *et al*. 1991, 1994; Pimenov *et al.* 1999) and has recently been discovered in the eastern Mediterranean (Salas & Woodside 2002; Olu-Le Roy *et al*. 2004). Associated chemosymbiotic species include Vestimentifera and Frenulata tube worms, (Polychaeta: Siboglinidae), Cladorhizidae and Hymesdesmiidae sponges and bivalves (Sibuet & Olu 1998; Levin 2005). The dominant bivalves at cold seeps are large chemosymbiotic species belonging to the families Vesicomyidae and Mytilidae with Thyasiroidea, Solemyoidea and Lucinoidea sometimes also abundant.

Thyasirids have a much wider distribution than other chemosymbiotic bivalve families; they are found from coastal to hadal depths, in different types of sediments, and from both the poles to the equator. They may show a wide variation in their anatomical characters and in the extent of their nutritional reliance upon symbionts (Dufour 2005). The generic definitions within the Thyasiroidea have been recognized to be problematic (Payne & Allen 1991; Oliver & Killeen 2002; Oliver & Sellanes 2005). Routinely, they can be separated into two groups: those with larger, upright (dorso-ventrally extended) shells and those with minute, elongate (antero-posteriorly extended) shells (Payne & Allen 1991). This is reflected in the degree of symbiosis where the former are chemosymbiotic and latter lack bacteria. Thyasirids, as opposed to many other bivalves with chemoautotrophic symbionts, are small (Dufour 2005), their bacteria are extracellular (exception of *Maorithyas hadalis* (see Fujiwara *et al.* 2001) and only within the Thyasiridae is there a genus, *Thyasira* (as currently defined), in which some species contain symbionts and others lack them (Southward 1986; Dufour

2005). Although chemosymbiotic species are widespread in reducing environments, relatively few are associated with chemosynthetic environments such as hydrothermal vents, methane seeps or whale falls (Oliver & Holmes 2006).

This paper reports on the Thyasiroidea recovered from mud volcanoes of the Gulf of Cadiz (Figure 2.2.1). Mud volcanoes discovered on the Moroccan margin were reported by Gardner (2001) and the first evidence of seep-related fauna in the Gulf of Cadiz was briefly mentioned by Pinheiro *et al*. (2003) from samples collected in mud volcanoes from the Moroccan, Spanish and deep Portuguese margins. Within the framework of the UNESCO/IOC Training Through Research Program (since 2000) and the EU-funded project HERMES (since 2005) samples were collected from over 20 mud volcanoes with varying seepage activity, at depths ranging 200 to 3900m. The first occurrence of thyasirids in the Gulf of Cadiz was reported from the Carlos Ribeiro mud volcano (Pinheiro *et al.* 2003) but it was only recently (during cruises TTR14 in 2004 and TTR16 and the MSM01-03 in 2006) that a large number of thyasirid specimens were collected mostly from two sites: Captain Arutyunov and Carlos Ribeiro mud volcanoes.

2.2.2.Materials and Methods

The material from the Gulf of Cadiz was collected during several TTR cruises (IOC-UNESCO) onboard the RV Prof. Logachev and the MSM01-03 (IFM-GEOMAR) cruise onboard the RV Maria S. Merian using a TV-assisted grab or a USNEL box-corer. Occasionally faunal specimens were also recovered from Kasten-corer, multiple corer or lander samples that were carried out for different purposes. Whenever possible the specimens were sorted on board and preserved in 70 or 96% ethanol (the latter preserved for molecular analysis). Most material is deposited in the Biological Research Collection of the Department of Biology, University of Aveiro but the holotype and paratypes of the new species are deposited in the National Museum of Wales and in the National Museum of Natural History, Washington DC, respectively.

During the MSM01-03 cruise it was also possible to collect thyasirid specimens for stable isotope analysis (station 157 GKG5; station 169 MUC7 and station 217 GKG10). During the TTR16, one live specimen (station AT615Gr) was dissected onboard and its tissue frozen for stable isotope analysis and molecular characterization of putative chemosynthetic prokaryotic endosymbionts. These results will be reported elsewhere.

The anatomical dissections were made following light staining in methyl blue to give contrast to the tissues for subsequent photography. Photography of the dissections and the shells was carried out using computer enhanced digital imaging with AutoMontage™ software. The terminology for shell structures and morphology follows that of Oliver & Killeen (2002).

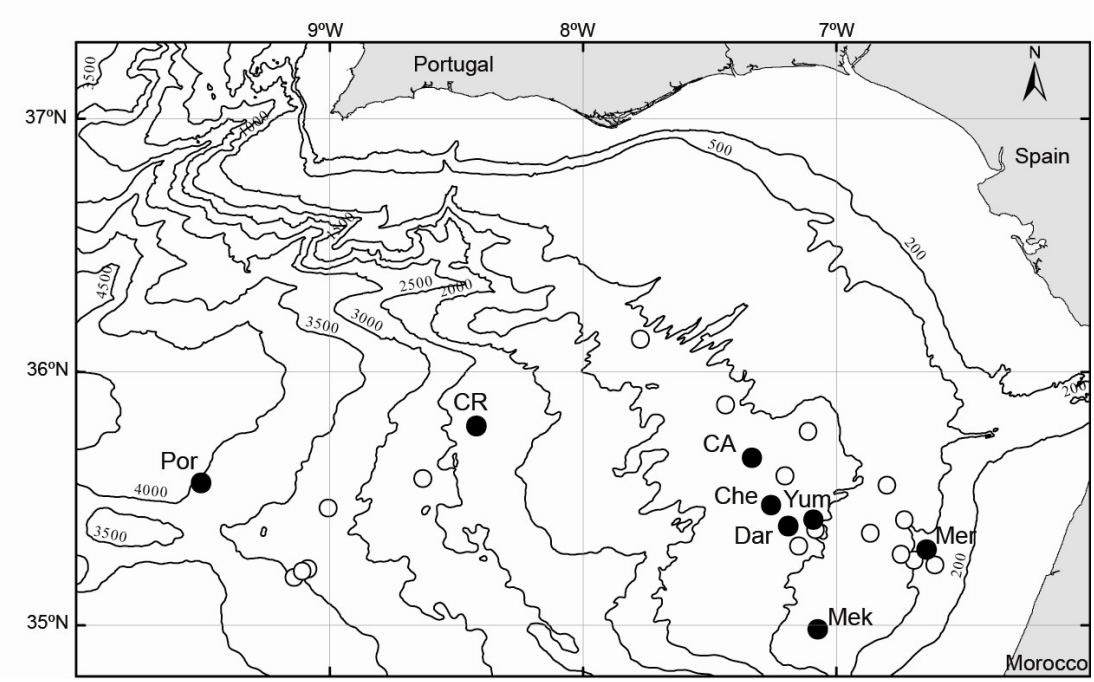

Figure 2.2.1. Map of the study area. Legend: Full circles, Mud volcanoes with Thyasiroidea: CA – Captain Arutyunov, CR – Carlos Ribeiro, Che – Chechaouen, Dar - Darwin, Mer – Mercator, Mek – Meknès, Por – Porto, Yum – Yuma; Empty circles, other mud volcanoes in the Gulf of Cadiz.

Abbreviations

Anatomical abbreviations. a, anus; **aa**, anterior adductor muscle; **apr**, anterior pedal retractor muscle; **ct**, ctenidium; **ddd**, digestive divertular duct; **dg**, digestive gland; **d lpb**, duct of lateral body pouch; **ex a**, exhalant aperture; **f**, foot; **g**, gonad; **h**, heart; **hg**, hind gut; **ifj**, inter filamental junction; **id**, inner demibranch; **in gl**, inhalant glandular arera; **k**, kidney; **lbp**,

lateral body pouch; **llbp**, duct to the left lateral body pouch; **lp**, labial palps; **m**, mouth; **me**, mantle edge; **mg**, midgut; **neck lpb**, neck of lateral body pouch; **od**, outer demibranch; **pa**, posterior adductor muscle; **p gl**, posterior glandular area; **ps**, posterior septum; **rdg**, ridge attached to sensory papilla; **rlbp**, duct to the right lateral body pouch; **sp**, sensory papilla; **s**, stomach; **vmf**, ventral mantle folds.

Institutional abbreviations. DBUA, Department of Biology, University of Aveiro (Biological Research Collection); IFM-GEOMAR, Institut für Meereskunde - Forschungszentrum für marine Geowissenschaften; IOC-UNESCO, Intergovernmental Oceanographic Commission – United Nations Educational, Scientific and Cultural Organization; NMW.Z, National Museum of Wales, Cardiff, Great Britain; USNM, [United States] National Museum of Natural History.

2.2.3.Systematics

Class Bivalvia

Order Veneroida

Superfamily Thyasiroidea Dall, 1900

Family Thyasiridae Dall, 1900

Genus Thyasira Leach *ms in Lamarck, 1818*

Type species. *Tellina flexuosa* Montagu, 1803

Generic Definition. Fragile shells, subcircular, ovate to ovate-polygonal in outline with a posterior sulcus; escutcheon variably expressed, absent to deep, with or without an auricle producing a submarginal sulcus. Hinge teeth lacking or as a single "cardinal" tubercle, ligament sunken. Anterior adductor scar elongate, posterior adductor scar ovate, pallial line entire. Ctenidium with two demibranchs, lateral body pouches large and multilobed, foot vermiform, heel obsolete, toe developed.

***Thyasira vulcolutre* sp. nov. Rodrigues CF & PG Oliver** (Figure 2.2.2- Figure 2.2.4)

Material examined. Holotype: one live-collected specimen, MSM01-03 stn 217 GKG10, Captain Arutyunov mud volcano, Gulf of Cadiz, 35º39.643'N, 07º20.046'W, 1321 m, 30 April 2006, M.R. Cunha (NMW.Z.2007.3.1).
Paratypes: 24 live-collected specimens as holotype. (NMW.Z.2007.3.2-3; USNM.1110226, DBUA00826.01).

Other material: All from Gulf of Cadiz; nine-live collected specimens, MSM01-03, stn 246/274 BIGO3, Captain Arutyunov mud volcano, 35º39.738'N, 07º20.010'W, 1321 m, 10 May 2006 (NMW.Z.2007.3.4-6, DBUA00826.02); one live-collected specimen, TTR10, stn AT243G, Carlos Ribeiro mud volcano, 35º47.217'N, 08º25.313'W, 2200 m, 26 July 2000 (DBUA00827.01); one shell, TTR16, stn AT615Gr, Carlos Ribeiro mud volcano, 35º47.238,N, 08º25.272'W, 2200 m (NMW.Z.2007.3.7).

Description. *Measurements: Holotype,* length 17.0 mm, height 16.5 mm, tumidity 10.4 mm. Summary statistics of morphometric ratios are presented in Table 2.2.1.
Shell (Figure 2.2.2: 2-11): To 17.2 mm in length; thin shelled; equivalve; subequilateral, beaks slightly in front of the midline, pointed prosogyrate. Relatively tumid but variable, (Length:Tumidity ratio 1.3:1 to 1.9:1, mean 1.6:1). Outline typically subcircular, with length similar to height, some specimens with length greater than height (see variation below). Lunule margin incurved, long. Anterior dorsal margin narrowly curved, ventral margin broadly curved. Posterior sinus distinct but shallow, submarginal sinus distinct. Posterior dorsal margin more or less straight. Posterior sulcus shallow but distinct, submarginal sulcus shallow but with a sharp, well-defined margin. Auricle elevated but low and very long almost filling the entire escutcheon. Ligament visible for most of the length of the auricle (Escutcheon:auricle ratio 1.1:1 to 1.5:1, mean 1.3:1). Lunule shallowly sunken, cordate, broad. Hinge plate narrow, lacking clearly defined teeth. Ligament long, sunken. Sculpture concentric, of lines and relatively regular narrow ridges. Radial lines visible in some, but are not surface features but are reflected inside the shell by

numerous radial striations. Pallial line entire. Anterior adductor scar long; posterior adductor scar oval. Periostracum very thin, pale, greyish brown. Variation: The outline is variable (Figure 2.2.2: 8-10), this variability generally increases with the size of shell, and is reflected mostly in the disproportionate increase in tumidity in the largest size classes. The expression of the lunule is highly variable and is probably related to the variation in shell tumidity.

Table 2.2.1. Shell dimension ratios for 21 shells from type series of *Thyasira vulcolutre* sp. nov.

	Mean	Std. Dev.	Count	Minimum	Maximum
Length/Height	0.99	0.04	21	0.93	1.11
Length/Tumidity	1.60	0.17	21	1.29	1.86
Length/Length lunule	3.49	0.39	21	2.81	4.11
Escutcheon/auricle	1.25	0.08	21	1.14	1.47

Anatomy: Mantle edge fused posteriorly between termination of gill axis and posterior adductor; exhalant siphonal opening simple, margins smooth (Figure 2.2.3: 13). Labial palps small, with indistinct sorting ridges. Posterior ventral mantle edge with a glandular area on its inner side (Figure 2.2.3: 16). Ventral mantle edge with a distinct median fold (Figure 2.2.3: 17). Anterior inhalant aperture with an elongate glandular area on its inner side (Figure 2.2.3: 14); ventral side of anterior adductor muscle with a digitate sensory papilla (Figure 2.2.3: 14, 15). Ctenidium large, of outer and inner demibranchs, both as thick fleshy lamellae (Figure 2.2.3: 12). Outer demibranchs about 2/3 size of inner demibranchs. Foot elongate, vermiform (Figure 2.2.3: 12), with a muscular ring at the junction with the visceral mass; heel indistinct. Visceral mass, lateral body pouches large, extensively lobed (Figure 2.2.4: 12, 18). Lobes flat-ended, roughly cuboidal. Lobes arise from large, single orifice at base of stomach (Figure 2.2.4: 19, 20). Stomach cylindrical in a transverse longitudinal orientation; midgut, a simple tight loop and lies over stomach before looping posteriorly into the
straight hindgut and rectum (Figure 2.2.4: 20). Kidneys large (Figure 2.2.4: 19), with dense aggregations of crystalline granules and a yellow/white fibrous mass in the lumen.

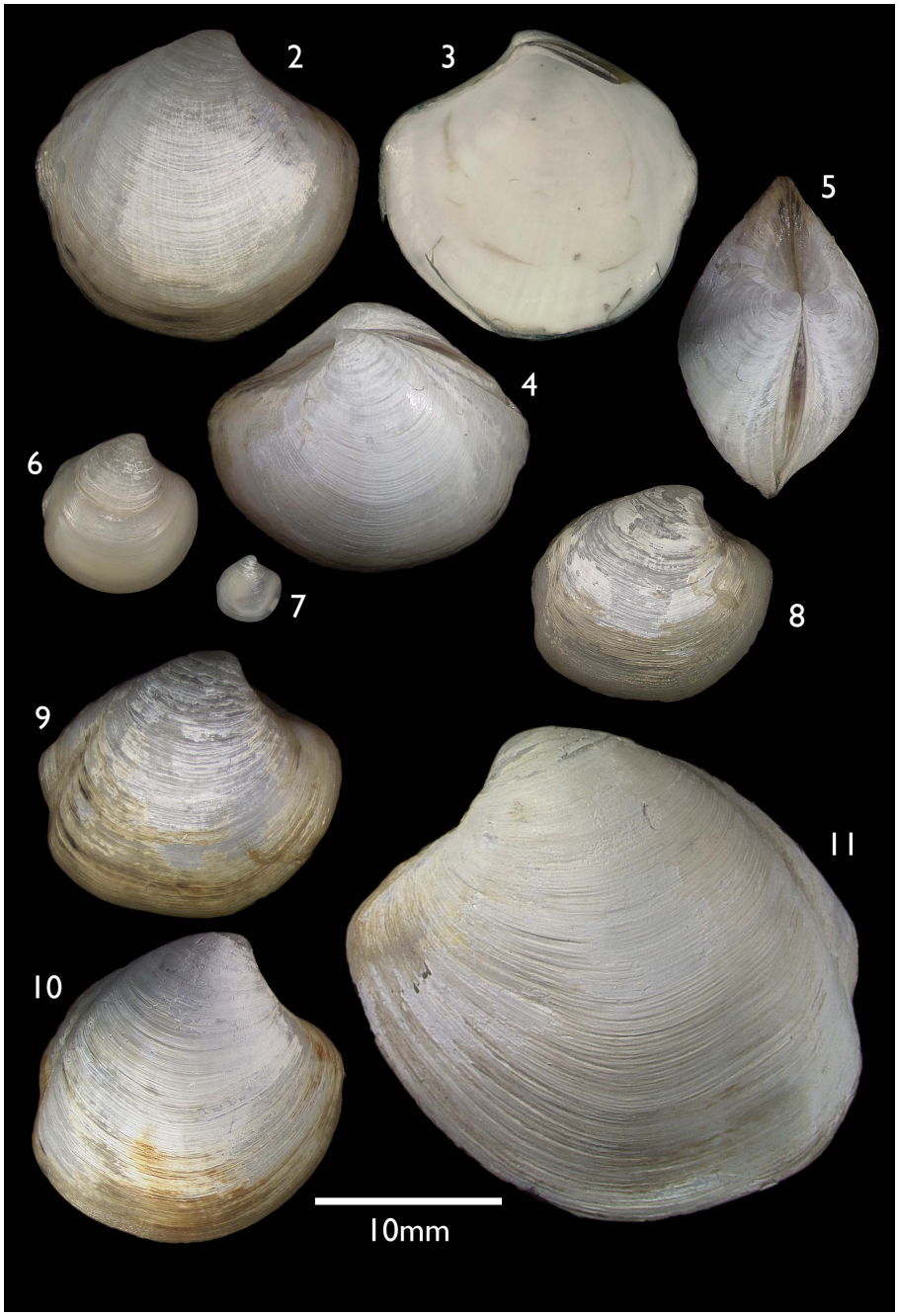

Figure 2.2.2. *Thyasira vulcolutre* sp. nov.; 2-5: Holotype, NMW.Z.2007.3.1; 6 & 7: Juvenile shells, NMW.Z.2007.3.2-3; 8-10: variations in shells form, NMW.Z.2007.3.4-6; 11: large shell, NMW.Z.2007.3.7.

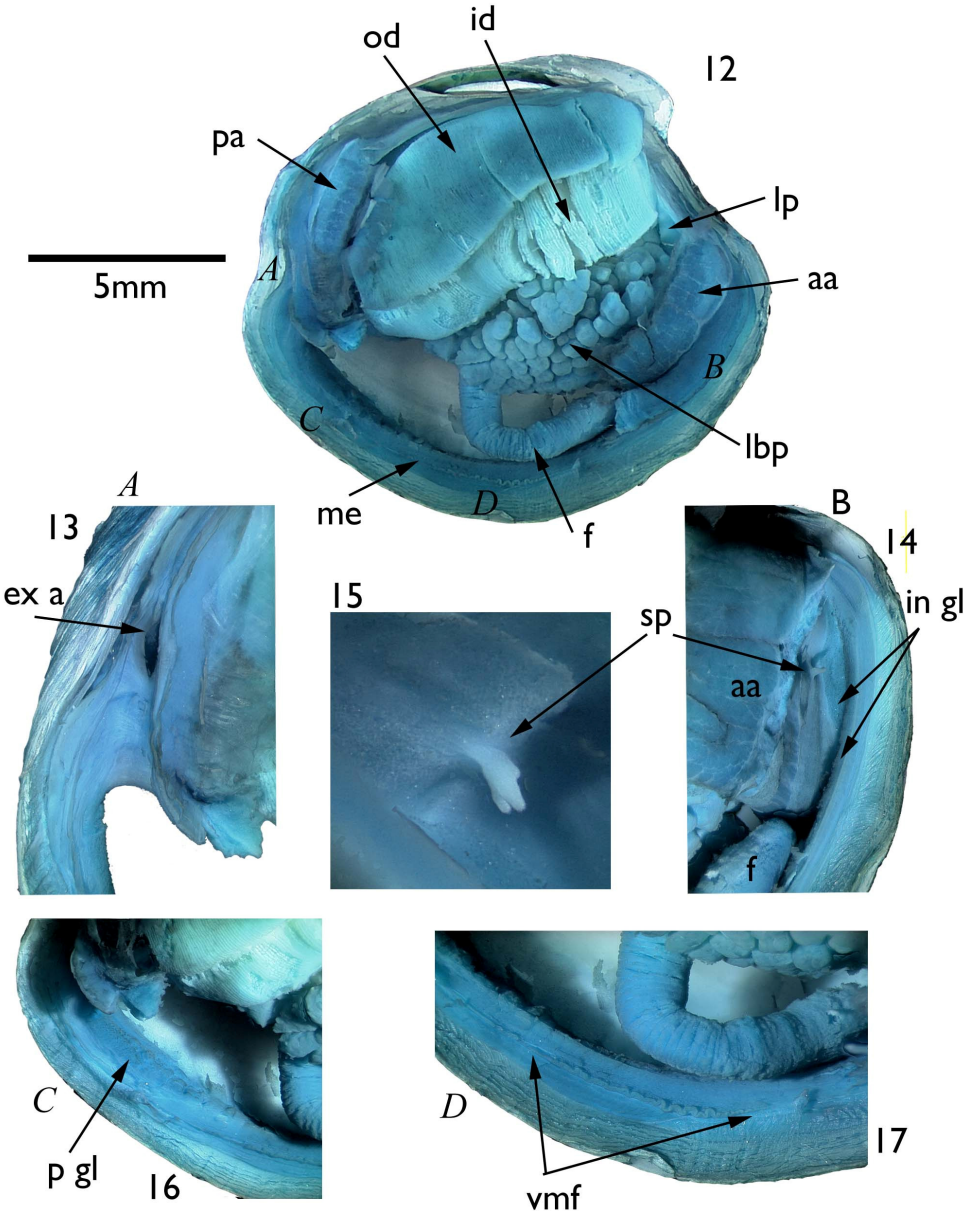

Figure 2.2.3. *Thyasira vulcolutre* sp. nov., NMW.Z.2007.3.4-6. Gross anatomy after removal of the right shell and mantle. 12: whole view; 13: enlarged view of area *A*, the posterior exhalant aperture; 14: enlarged view of area *B*, the anterior inhalant aperture; 15: detail of the sensory papilla; 16: enlarged view of area *C*, the posterior ventral area; 17: enlarged view of area *D*, the median ventral mantle edge.

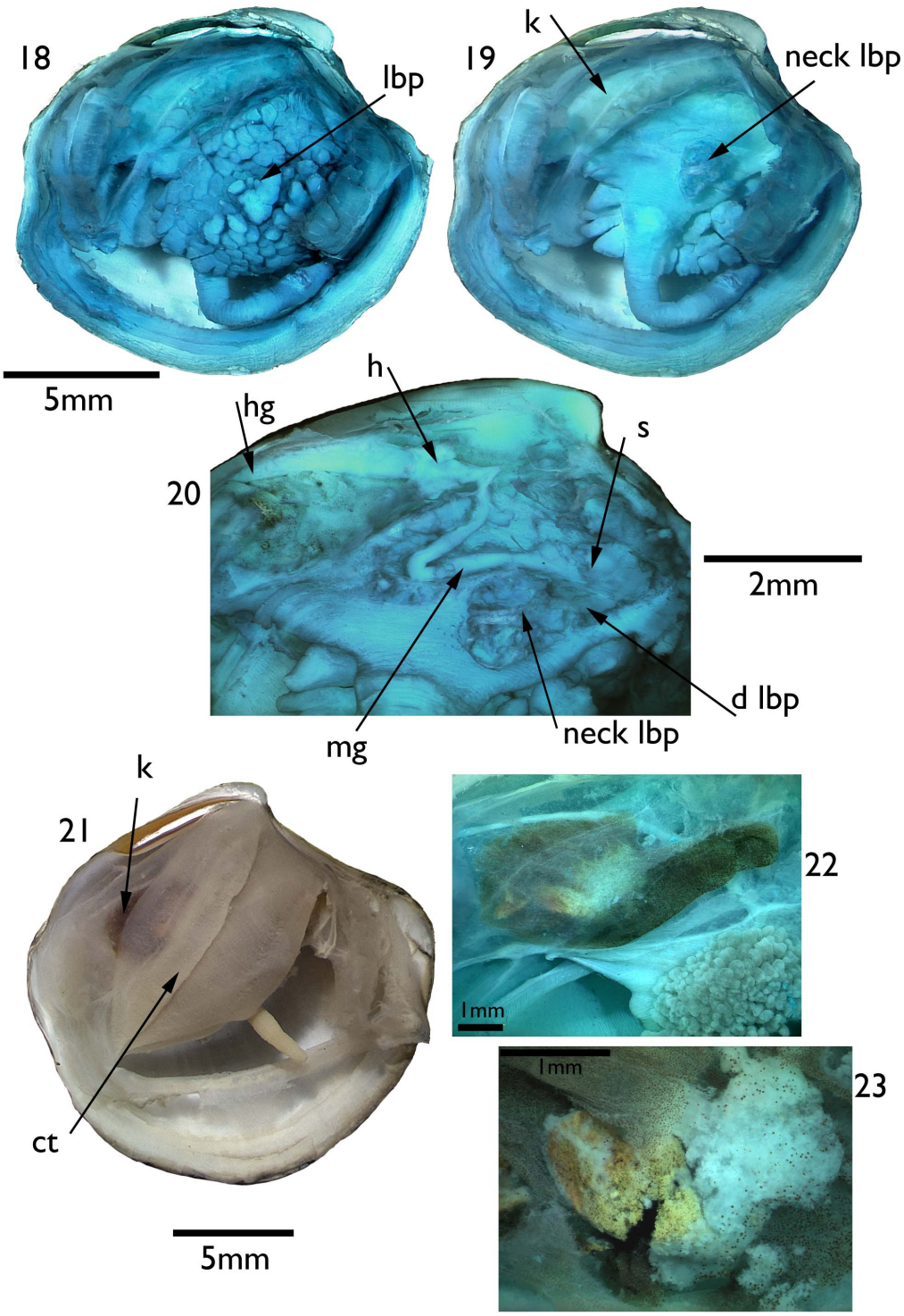

Figure 2.2.4. Figures 18-20: *Thyasira vulcolutre* sp. nov., NMW.Z.2007.3.4-6. 18: Gross anatomy after removal of right shell, mantle and ctenidium; 19: Gross anatomy after removal of right mantle, ctenidium and lateral body pouch; Fig 20: Dissection of the alimentary system. 21-23: *Thyasira vulcolutre* sp. nov. Holotype, NMW.Z.2007.3.1; 21: Gross anatomy after removal of right shell and mantle. 22: kidney as viewed through the epithelial tissues. 23: Kidney dissected showing crystalline granules and fibrous mass in lumen.

Note. Dissection of the holotype revealed an identical gross structure to that described above but the ctenidia were thin and semi-transparent and the lateral body pouches were greatly contracted (Figure 2.2.4: 21). The kidney was extensively stained dark brown and was massively infested with a fibrous mass and crystalline granules (Figure 2.2.4: 22, 23).

Distribution. Most of the specimens were collected on the Captain Arutyunov mud volcano, some specimens were also collected on Carlos Ribeiro mud volcano; depth range between 1300 and 2200m.

Etymology. Vulcolutre, Latin combination of vulcano from "volcano", lutum from "mud"; suffix tre denoting "belonging to".

***Remarks*.** Species comparisons: This new species is morphologically similar to *Thyasira sarsi* (Philippi, 1845), *T. southwardae* Oliver & Holmes, 2006*, T. oleophila* Clarke, 1989 *and T. methanophila* Oliver & Sellanes, 2005 all known to be associated with chemosynthetic sites. *Thyasira striata* Sturany, 1899 has also been recorded from mud volcanoes in the eastern Mediterranean (Olu-Le Roy *et al*. 2004) but that species has a very distinct posterior sulcus and is more similar to the *T. flexuosa* group than to the above taxa.

Thyasira sarsi has been reported from methane seeps in the North Sea (Dando *et al.* 1994) but is also known from vegetation-derived organic-rich environments in Norwegian fjords (Southward 1986). *Thyasira sarsi* differs by having a weakly developed posterior sulcus, a submarginal sulcus without a sharp, clearly defined margin and the lunule not so depressed and heart-shaped. *Thyasira sarsi* also possesses shell sculpture without concentric ridges and with a distinctly granular surface (see Oliver & Holmes 2006).

Thyasira southwardae has been reported only from the Logatchev hydrothermal vent in association with *Bathymodiolus* and *Calyptogena*. Only two shells and one valve of this species have been collected, making comparisons difficult but it is clear that the shells of *T. vulcolutre* and *T. southwardae* are similar. The shells of *T. southwardae* (see Oliver & Holmes 2006) are thinner and have irregular sculpture of concentric lines and corrugations and never display the regular concentric ridges typical of *T. vulcolutre*. The definition of the submarginal sulcus is not so sharp in *T.*

southwardae and the submarginal sinus and posterior sinus are not so pronounced. From the anatomy, the gills of *T. southwardae* were pink in contrast to the light brown in *T. vulcolutre*.

Thyasira oleophila was described from the Louisiana oil and gas seeps at depths around 500m. It is a large species reaching 22.0mm in length and is almost devoid of a posterior sulcus, is not expanded anteriorly and has a strongly granular surface (see Oliver & Holmes 2006).

Thyasira methanophila Oliver & Sellanes, 2005 was described from a methane seepage area off Concepcion, Chile at bathyal depths. *Thyasira methanophila* differs from *T. vulcolutre* in that the lunule is not sunken, and the escutcheon is less defined.

From morphology alone, a new genus could be proposed for the group of species similar to *T. vulcolutre,* including *T. methanophila, T. southwardae, T. sarsi and T. oleophila* but this is not wholly supported by the molecular data.

An 18S rRNA gene Neighbour Joining tree (not shown) combining an unpublished sequence from *Thyasira vulcolutre* with previously published sequences from thyasirids analyzed by Taylor, Williams and Glover (2007) shows that *T. vulcolutre* is clustered with *T. perplicata* Salas, 1996 and is distant from *T. methanophila* and *T. sarsi*. (S Williams & JD Taylor pers. comm.). Payne and Allen (1991) described *T. perplicata* under the name *T. excavata plicata* (Verrill, 1885) as having a shell with a strong posterior carina and thus most similar to shells of the genus *Conchocele*. Anatomically *T. perplicata* is also similar to *Conchocele* with a bulbous tipped foot, large fleshy gills and prominent anterior mantle edge folds (Nakazima 1958; Kamenev *et al*. 2001).

The lack of congruence between morphology and molecular data may be clarified once a larger set of taxa are included but until this study is completed the genus *Thyasira* is retained for the majority of species with sulcate shells, with two demibranchs and that are chemoautotrophic.

Functional morphology: The functional morphology is essentially similar to other thyasirids that are primarily chemosymbiotic and strongly associated with chemosynthetic settings (Oliver & Sellanes 2005). The gills are thick and fleshy and of Dufour Type 3 (Dufour 2005). The foot is vermiform and the

lateral body pouches are large and multilobed. Stable isotopic ratios are in the range of other symbiotic chemosynthetic-based bivalves hosting sulphide-oxydizing symbionts (CF Rodrigues, unpublished results).

The presence of a sensory papilla situated on the anterior ventral face of the anterior adductor muscle was first reported by Payne and Allen (1991) for the members of genus *Axinus*. A similar papilla is present in *A. cascadiensis*, and it was also reported on the dorsal surface of the adductor muscle in *T. sarsi*, *T. flexuosa*, *T. obsoleta* and *Axinulus croulinensis* (Oliver & Holmes i*n press*). In *Thyasira vulcolutre* the papilla (Figure 2.2.3: 14, 15) is situated in the inhalant flow and it probably functions in a sensory manner to monitor flow or water chemistry of the inhalant current.

An obvious feature of *Thyasira vulcolutre* is the extensive staining of the kidneys by both crystalline granules and fibrous material in the lumen. Similar deposits were seen in *T. methanophila* and could be related to metabolism in a mineral-rich environment or to the bacterial source of nutrition.

The unusual condition of the holotype suggests that the bacteria were lost from the gills and that digestion had slowed or ceased resulting in morbidity. The mortality of bivalves has been already studied and sulphide deprivation, sulphide toxicity, and disease were indicated as possible agents of mortality on the system (Mills *et al.* 2005).

Thyasira (Parathyasira) granulosa **(Monterosato, 1874)** (Figure 2.2.5: 24 & 25)

Material examined. All from Gulf of Cadiz; two live-collected specimens, MSM01-03, stn 241 GKG12, Mercator mud volcano, 35º17.918'N, 06º38.717'W, 353m, 6 May 2006 (DBUA00828.01); one live specimen MSM01-03, stn 242 GKG13, same locality, 35º17.870'N, 06º38.810'W, 350m, 6 May 2006 (DBUA00828.02); one live-collected specimen, MSM01-03, stn 321 GKG22, Meknès mud volcano, 34º58.796'N, 07º04.394'W, 732m, 14 May 2006 (DBUA00828.03); one live-collected specimen, TTR16 cruise, station AT 610Gr, Chechaouen mud volcano, 35º28.468N, 07º15.477W, 1177m, 30 May 2006 (DBUA00829.01).

Remarks. This species is readily identified by the radially arranged granular striations on the shell surface. It is widely distributed in the Mediterranean and northeastern Atlantic from 100 to 1800 m (Oliver & Killeen 2002). Dufour (2005) classified this as having Type 2 gills with both demibranchs present but lacking bacterial symbionts.

Thyasira obsoleta (Verrill & Bush, 1898) (Figure 2.2.5: 26)

Material examined. All from Gulf of Cadiz; two live-collected specimens, MSM01-03, stn 145 GKG3, Porto mud volcano, 35º33.699'N, 09º30.437'W, 3860m, 21 April 2006 (DBUA00831.01); one live-collected specimen MSM01-03, stn 190 MUC8, Captain Arutyunov mud volcano, 35º39.665'N, 07º19.970'W, 1322m, 28 April 2006 (DBUA00831.02); one live-collected specimen, MSM01-03, stn 241 GKG12, Mercator mud volcano, 35º17.918'N, 06º38.717'W, 353m, 6 May 2006 (DBUA00831.03); one live-collected specimen MSM01-03, stn 169 MUC7, Carlos Ribeiro mud volcano, 35º47.256'N, 08º25.361'W, 2199m, 25 April 2006 (DBUA00831.04).

Remarks. All specimens are very small (not exceeding 2.0mm) but exhibit the characteristically weakly sulcate posterior and angular outline. This is a common and widespread species recorded from across the Atlantic Ocean in depths from 24 to 2900m (Payne & Allen 1991). It has not been associated with any particular reducing environment. Dufour (2005) classified this as having Type 2 gills with both demibranchs but showing no evidence of bacterial symbiosis.

Thyasira tortuosa (Jeffreys, 1881) (Figure 2.2.5: 27)

Material examined. One live-collected specimen, TTR16 cruise, station AT 622Gr, Porto mud volcano, Gulf of Cadiz, 35º33.773N, 09º30.416W, 3902m, 3 June 2006 (DBUA00830.01).

Remarks. The only specimen examined is provisionally identified as *Thyasira tortuosa* and has the characteristically compressed and anteriorly expanded outline noted by Payne and Allen (1991). The shell from Gulf of Cadiz (5.5mm) is much larger than any of the shells described by these authors (3.2mm) and

comes from a greater depth: 3902m whilst the maximum given by Payne & Allen (1991) was 2359m. Dufour (2005) did not examine this species but the Gulf of Cadiz specimen has a Type 2 gill with both demibranchs. The gills are rather thick and have the same appearance as the ones that exhibit strong bacterial symbiosis such as *T. flexu*osa and *T. gouldi*.

***Axinulus croulinensis* (Jeffreys, 1847)** (Figure 2.2.5: 28)

Material examined. All from Gulf of Cadiz; twelve live-collected specimens, MSM01-03, stn 241 GKG12, Mercator mud volcano, 35º17.918'N, 06º38.717'W, 353m, 6 May 2006 (DBUA00832.01); four live-collected specimens, MSM01-03, stn 242 GKG13, same locality, 35º17.870'N, 06º38.810'W, 350m, 6 May 2006 (DBUA00832.02); one live-collected specimen, TTR15, stn AT576B, same locality, 35º17.657N, 06º39.129W, 428m, 26 July 2005 (DBUA00833.01).

Remarks. *Axinulus croulinensis* is recognised by the almost symmetrical oval outline and weak to absent posterior sulcus. It is widely distributed throughout the Atlantic Ocean. It has been recorded from 40 to 3800m and has not been associated with any specific reducing environment (Payne & Allen 1991). Dufour (2005) classified this as having Type 2 gills with single demibranchs and showing weak bacterial symbiosis.

***Mendicula ferruginosa* (Forbes, 1844)** (Figure 2.2.5: 29)

Material examined. Nine live-collected specimens, TTR15, stn AT575B, Mercator mud volcano, Gulf of Cadiz, 35º17.903N, 06º38.715W, 355m, 26 July 2005 (DBUA000834.01).

Remarks. The heavily encrusted ferruginous deposit and subovate outline are characteristic of this ubiquitous species. It has been recorded from many parts of the Atlantic and Mediterranean in depths from 50 to 4825m and has not been associated with any specific reducing environment (Payne & Allen 1991). Dufour (2005) classified this as having Type 2 gills with a single demibranch and showing no evidence of bacterial symbiosis.

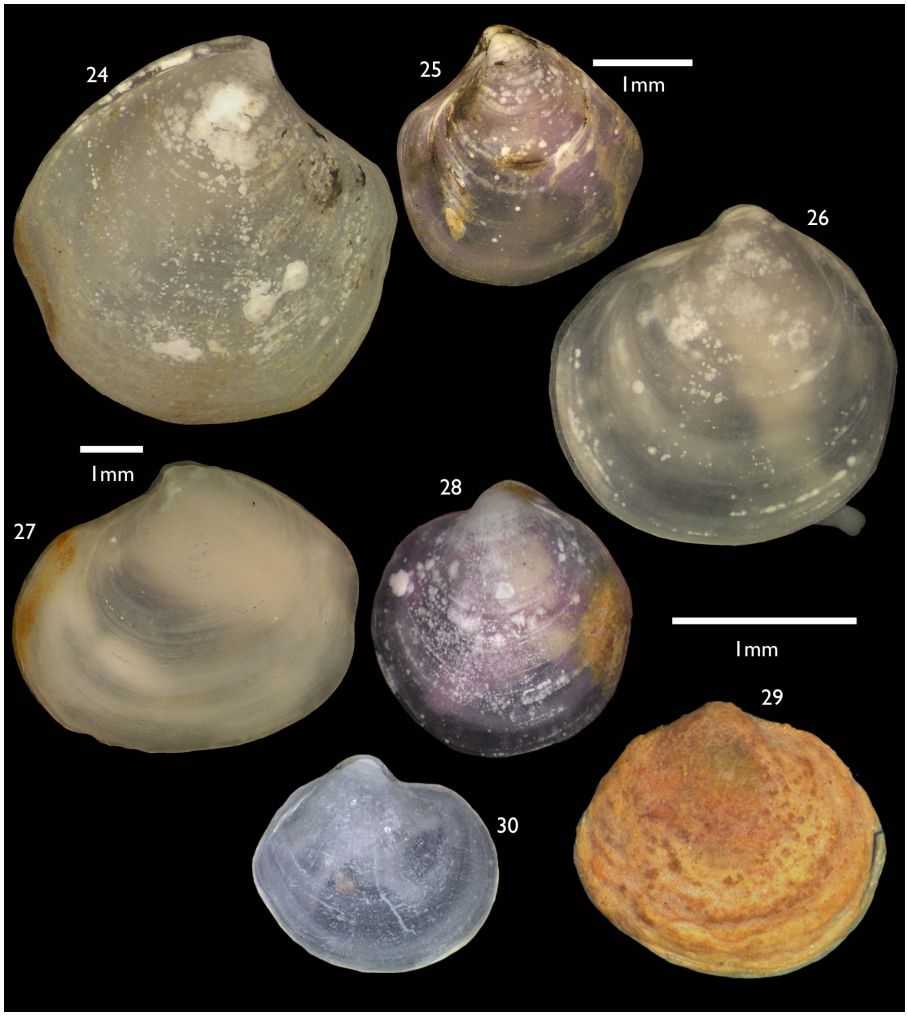

Figure 2.2.5. 24-25: *Thyasira (Parathyasira) granulosa* (Monterosato, 1874), 24 stn 321 GKG22, Meknès mud volcano, 732 m (DBUA00828.03), 25 stn 242 GKG13, Mercator mud volcano, 350 m (DBUA00828.02); 26 *Thyasira obsoleta* (Verrill & Bush, 1898) stn 190 MUC8, Captain Arutyunov mud volcano, 1322 m, (DBUA00831.02); 27 *Thyasira tortuosa* (Jeffreys, 1881) AT 622Gr, Porto mud volcano, 3902 m (DBUA00830.01); 28 *Axinulus croulinensis* (Jeffreys, 1847) stn 242 GKG13, Mercator mud volcano, 350 m (DBUA00832.02); 29 *Mendicula ferruginosa* (Forbes, 1844) stn AT575B, Mercator mud volcano, 355 m (DBUA000834.01); 30 *Leptaxinus minutus* Verrill & Bush, 1898, stn AT608Gr, Darwin mud volcano, 1115 m (DBUA00836.01).

***Leptaxinus minutus* Verrill & Bush, 1898** (Figure 2.2.5: 30)

Material examined. All from Gulf of Cadiz; two live-collected specimens, MSM01-03, stn 242 GKG13, Mercator mud volcano, 35º17.870'N, 06º38.810'W, 350m, 6 May 2006 (DBUA00835.01); one live-collected specimen, TTR16, stn AT608Gr, Darwin mud volcano, 35º23.531N,

07º11.475W, 1115m, 30 May 2006 (DBUA00836.01); two live-collected specimens, TTR16, stn AT604Gr, Yuma mud volcano, 35º25.820N, 07º06.330W, 1030m, 29 May 2006 (DBUA00836.02).

Remarks. *Leptaxinus minutus* is rather tumid with a distinctly angular posterior outline but lacking a posterior sinus. The hinge is well developed with weak lateral folds and a tubercular cardinal. It was described from the northwest Atlantic and has been recorded from Norway and Iceland (Ockelmann in Oliver & Killeen 2002). The Gulf of Cadiz specimens agree well with the shell figured by Oliver and Killeen (2002, Pl.25 C, D) but comes from a much greater depth. Dufour (2005) did not examine this species but the Gulf of Cadiz specimens have a Type 2 gill with a single demibranch and according to similar forms described by this author, it will doubtfully be symbiotic.

2.2.4.Ecological setting

Thyasirids have been found in hydrothermal vents (Gebruk *et al.* 2000; Oliver & Holmes 2006), cold seeps (Clarke 1989; Lewis & Marshall 1996; Imhoff *et al*. 2003; Olu-Le Roy *et al.* 2004; Oliver & Sellanes 2005), and oxygen-minimum zones (Oliver & Levin 2006). Although many of the larger Thyasiroidea species can be found at bathyal depths, most of the symbiotic species studied by Dufour (2005) live at somewhat shallower depths. Furthermore, among asymbiotic thyasirids, most of the deep-sea dwelling species were small and had only one demibranch (Dufour 2005). The larger size of chemosynthetic species could be due to a greater nutritional input than strict suspension-feeders, especially in the deep-sea, where particulate food is less abundant (Dufour 2005).

The Gulf of Cadiz, located west of the Strait of Gibraltar, is the zone of exchange between the NE Atlantic Ocean and the Mediterranean Sea. The Mediterranean inflow/outflow strongly influences the local circulation features particularly due to the generation of the intermediate depth (750-1250m) Mediterranean Outflow Water mass (MOW) and Mediterranean water eddies. Concerning the extra-coastal dynamics, the Gulf of Cadiz is also under the influence of the eastern end of the cross-Atlantic zonal jet. (Peliz *et al.* 2006).

Geologically, the setting of the study area is still under debate (Gutscher *et al.* 2002; Medialdea *et al*. 2004; Duarte *et al*. 2005) but one of the most important structures is a thick (more than 5 km) Miocene-Pliocene sedimentary sequence emplaced on the structurally complex convergent tectonic setting of the African and Eurasian plate boundary (Hensen *et al.* 2007). Mud volcanism is triggered mainly by the compressional stress along this boundary. Fluid geochemistry indicates a deep thermogenic source of the fluids caused by clay mineral dehydration followed by fluid mobilisation along deeply rooted fault systems (Van Rensbergen *et al*. 2005; Hensen *et al*. 2007). The fluids on average are highly enriched in methane but concentrations in the upper 30 cm layer of the sediment vary widely from site to site, and even locally (e.g. Nuzzo *et al*. 2005). However, the sulphide/methane gradient is usually located at more than 30 cm depths in the sediment and methane concentrations at the sediment/water interface are usually very low.

The Gulf of Cadiz is presently the most extensive cold seepage area known from the European margins spreading between 200 and 4000 m depths and including over 30 mud volcanoes. To date, 27 mud volcanoes have been studied, but biological samples are available from only 18 and only on eight were thyasirids found: Mercator (355 m depth), Meknès (706m depth), Yuma (975m depth), Darwin (1115m depth), Chechaouen (1177m depth), Captain Arutyunov (1346m depth), Carlos Ribeiro (2200 m depth) and Porto (3902m depth).

Mercator is one of the shallowest mud volcanoes from the El Arraiche field in the Moroccan margin and it differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water. The top of the structure shows disturbed sediments with clasts and rock/carbonate blocks, small patches of exposed greenish mud breccia and larger patches of coarse sediments and shell debris. In one of those patches gas venting was observed during TTR15 and confirmed at the exact same location during the MSM01-03. Samples collected in this area yielded lucinid and solemyid bivalves while several thyasirid species (*Axinulus croulinensis, Leptaxinus minuta*, *Thyasira granulosa, Thyasira obsoleta* and *Mendicula ferruginea*) were collected mostly

from the central area of the mud volcano characterised by the occurrence of a field of solitary corals, accompanied by Cidaridae echinoids and Onuphidae polychaetes (*Hyalinoecia tubicola* (Müller)). The mud volcano is also colonized by *Isidella elongata* (Esper), cerianthids, other cnidarians, large sponges and abundant mobile megafauna, especially fish and shrimps.

Meknès is the southernmost Moroccan mud volcano rising among an extensive field of small coral mounds. The crater is formed by stiff, sometimes heavily disturbed, green mud breccia with scattered clasts and a striking large number of empty shells of the gastropod *Neptunea contraria* (Linné), in patches of a few meters in diameter. Living megafauna is rarely sighted. A small carbonate mound formed by an intricate network of coral debris and cemented sediments is located southwards of the main crater. *Thyasira granulosa* was collected from the top of this mound in a patch of heavily disturbed sediment with abundant solemyid shell debris.

Yuma, Darwin and Chechaouen are located in the western Moroccan field where extensive *Bathymodiolus* shell fields suggest that this was a very active seepage area in the past. Yuma is a complex mud volcano with three craters. *Leptaxinus minutus* Verrill & Bush, 1898 specimens were collected from an area in the northern slope showing abundant biological debris (scattered *Neptunea contraria* shells and *Bathymodiolus* graveyards) and some burrows but poorly colonized by megafauna (except for some shrimps and a few fish). At Chechaouen, *Thyasira granulosa* was collected from the crater among scattered crusts, clasts and small dead coral colonies inhabited by a few soft corals and shrimps. The top of Darwin mud volcano was covered by large carbonate slabs and crusts. The fissures among slabs and depressions with scattered crust were filled by abundant shells (*Bathymodiolus* and *N. contraria*) and occasionally small clumps of living *Bathymodiolus*. A successful attempt to sample one such clump was made and together with the *Bathymodiolus* a few specimens of *Leptaxinus minutus* were retrieved.

Captain Arutyunov, Carlos Ribeiro and Porto are aligned along major crustal strike-slip faults associated with the African-Eurasian plate boundary (Duarte *et al*. 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations measured at Captain Arutyunov and Carlos

Ribeiro yielded the highest records from the Gulf of Cadiz. The top of Captain Arutyunov appeared highly active with a rim of disturbed sediment and numerous Buccinidae shells and small clasts. Towards the centre the seafloor was rippled, covered by numerous clasts and shells and had a patchy appearance. The most conspicuous megafauna were Cidaridae echinoids clustered at the centre of the mud volcano. *Thyasira vulcolutre* specimens and one specimen of *T. obsoleta* were collected from several samples taken at the top together with a high density assemblage of Siboglinidae and Ampharetidae polychaetes, and tube-building tanaids. Most specimens were collected from the sample GKG10 where the sediment was heavily disturbed by the abundant methane bubbling and gas hydrates dissociation. At Carlos Ribeiro the megafauna assemblage was diverse at the flanks and dominated by echinoderms (ophiuroids, stalked crinoids, aggregations of Holothuria) and cnidarians. The top of the mud volcano was almost devoid of megafauna with the exception of scattered ophiuroids, a few burrows and scattered empty shells at the surface of the sediment. Black tubes of Siboglinid worms (A. Hilário pers. comm.) were present but difficult to see in the video images. Thyasirids (*T. vulcolutre* and *T. obsoleta*) were collected from the crater. The sampled sediments were soupy and expanded, clearly indicating the presence of dissociating gas hydrates. The top of Porto is covered by a continuous field of Siboglinidae tubeworms arranged in clumps. The tubeworms are sufficiently large to be seen in the video images and are accompanied mostly by stalked hexactinellid sponges and some crinoids; many old tubes and sponge stalks are colonized by epifaunal organism. Mobile fauna (galatheid lobsters, ophiuroids), feeding traces and scattered solemyid shells were also observed among the tubes. *Thyasira tortuosa* and *T. obsoleta* were collected together with clumps of several siboglinid tubeworm species.

The distribution of thyasirids in general shows little pattern in relation to individual mud vulcanoes. Only *Thyasira vulcolutre* shows a strong association to active seeps and this is the only species recovered with a "likely" high dependence on chemosymbiosis. Of the other taxa only *Axinulus croulinensis* has bacterial symbionts but in small numbers (Dufour 2005). All taxa except *T. vulcolutre* have been previously found in non-chemosynthetic settings and

are, in general, typical of deep-water benthic communities (Payne & Allen 1991). The number of specimens collected also rather low despite the careful sampling using box cores and a 0.5mm sieve. Again, only *T. vulcolutre* was found in high numbers contrasting with the other species and suggesting that the faunas, at least for thyasirids, are impoverished except at active sites or where animals can reach residual sulphide or methane sources. A more wide ranging faunistic analysis will hopefully reveal more meaningful distributional patterns.

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Chapter 2.3.Chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz, with descriptions of new species of Solemyidae, Lucinidae and Vesicomyidae

Abstract

The chemosymbiotic bivalves collected from the mud volcanoes of the Gulf of Cadiz are reviewed. Of the ten species closely associated with chemosynthetic settings two Solemyidae, *Petrasma elarraichensis* sp. nov. and *Acharax gadirae* sp. nov., one Lucinidae, *Lucinoma asapheus* sp. nov., and one Vesicomyidae, *Isorropodon megadesmus* sp. nov. are described and compared to close relatives of their respective families. The biodiversity and distribution of the chemosymbiotic bivalves in the Gulf of Cadiz is discussed and compared to the available information from other cold seeps.

Oliver PG, **Rodrigues CF** & Cunha MR (in preparation) Chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz, with descriptions of new species of Solemyidae, Lucinidae and Vesicomyidae. To be submitted to ***Journal of Molluscan Studies***.

Note: Since this paper is not yet submitted all the names should be considered provisional as they require validation through peer-reviewed publication

Chemosymbiotic bivalves from the mud volcanoes of the Gulf of Cadiz, with descriptions of new species of Solemyidae, Lucinidae and Vesicomyidae

2.3.1.Introduction

There are five major bivalve families with representatives among the typical symbiont-bearing macroinvertebrates in seep assemblages: the Solemyidae, Lucinidae, Vesicomyidae, Thyasiridae and Mytilidae (Sibuet & Olu 1998; Sibuet & Olu-Le Roy 2002; Sahling *et al.* 2003; Levin 2005). The occurrence of chemosymbiotic bivalves in the extensive mud volcano fields of the Gulf of Cadiz was first reported by Pinheiro *et al*. (2003) followed by more in-depth studies on polychaete commensals of solemyid hosts (Ravara *et al*. 2007), on the distribution and taxonomy of Thyasiridae (Rodrigues *et al*. 2008), on the phylogenetic relationships of *Bathymodiolus mauritanicus* (Génio *et al*. 2008) and on the molecular characterization of chemosymbiotic endosymbionts of solemyids, lucinids and mytilids (Rodrigues *et al*. submitted). In this paper two new species of Solemyidae, one new species of Lucinidae and one new species of Vesicomyidae are described and the information on the distribution of chemosymbiotic bivalves in the Gulf of Cadiz is updated and discussed.

Solemyids are among the most primitive bivalves dating from the Paleozoic (Métivier & Cosel 1993). The ancestral protobranch molluscs are a small, remarkably uniform group of marine bivalves with a pratically worldwide distribution (Conway *et al*. 1992). They are characterized by an elongate shell (up to 90mm) with a strongly posteriorly situated toothless hinge and thick brown periostracum that is much larger than the calcified part of the valve and that is folded inwards by the muscular mantle edge upon closing of the valves (Métivier & Cosel 1993). Solemyidae taxonomy is complex. Three extant genera, *Solemya* Lamarck 1908, *Acharax* Dall 1908 and *Petrasma* (Taylor *et al*. 2009) are currently recognized. The genus *Solemya* is generally found at continental-shelf and upper-slope depths (0 to 600m), while the genus *Acharax* is found deeper, at depths from ~400m on the continental slope to

the deepest sites of the Japan Trench (reviewed by Neulinger *et al*. 2006). The genus *Solemya* lives in sediments with high organic-matter content, often at reduced oxygen concentrations. Hydrogen sulphide is frequently present due to sulphate reduction coupled with organic matter degradation (Conway *et al*. 1992). In contrast, the genus *Acharax*, which lives buried in reducing sediments, has rarely been taken alive, and information on its phylogenetic position and diversity is lacking and only now several studiesstart arising Recovery or observations of the characteristic thick periostracum was often the only evidence for the presence of this genus (Neulinger *et al*. 2006). The genus *Acharax* was also found at other cold seep locations (see review by Sibuet & Olu 1998) and in sediments influenced by hydrothermal venting (Juniper *et al*. 1992; Métivier & Cosel 1993). However, at a first glance both solemyids appear so similar that specimens discovered at various deep-sea sites might have been misclassified erroneously as *Solemya* (see review by Sibuet & Olu 1998).

The Lucinidae, in which the sulphide-oxidizing symbiosis is likely obligate, is by far the most disparate and species-rich family. Habitats occupied by lucinids range from the intertidal zone to depths of over 2100m. Some species are associated with cold seeps and mud volcanoes, oxygen minimum zones and a single species is knon from a hydrothermal vent (reviewed by Taylor & Glover 2006). Shells of Lucinidae are usually white, subcircular in outline and range from discoidal to subspherical in profile. Living species range in height from around 3−140mm. External sculpture mainly comprises variations of commarginal lamellae, radial ribbing is usually absent or subordinate and posterior sulci are often present (Taylor & Glover 2006).

The family Vesicomyidae includes highly specialized bivalves known worldwide from continental slopes to hadal depths. Most species of the family inhabit reducing sediments, predominantly of muddy consistence, and many are found in subduction areas, around hydrothermal vents (Boss & Turner 1980; Tunnicliffe 1991) or in cold seep areas (Turner 1985; Okutani & Métivier 1986). Despite their conspicuous presence in many reducing environments, the taxonomy of vesicomyids is far from being settled, both at species and supraspecific levels (Krylova & Sahling 2006). Different authors estimate that

the family includes from 50 to more than 70 recent and fossil species (reviewed by Krylova & Sahling 2006). Their size ranges from very small (*Vesicomya atlantica*) to very large specimens (*Calyptogena magnifica*) (Boss & Turner 1980). Until now eleven species have been reported from the eastern Atlantic (reviewed by Cosel & Salas 2001).

2.3.2.Material and Methods

Study area. The Gulf of Cadiz is located in the NE Atlantic Ocean between 34ºN and 37º15'N and 6ºW to 9º45'W. It is enclosed by the southern Iberian and northern Moroccan margins, west of Gibraltar Strait. The geological history of the Gulf of Cadiz is intimately related to plate tectonic interaction between Southern Eurasia and North Africa and is driven by two major mechanisms: a) subduction associated with the westward emplacement of the Gibraltar Arc and formation of the Gulf of Cadiz accretionary wedge, probably not active at present and b) oblique lithosphere collision between Iberia and Nubia, active at present and causing active thrusting (Zitellini *et al*. 2009). It is now well established that the whole area is under compressive deformation and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages. This extensive area encompasses over forty mud volcanoes (confirmed by coring), at depths ranging from 200 to 4000m (Mazurenko *et al*. 2002; Pinheiro *et al*. 2003; Magalhães 2007), and active methane seepage has been documented on several locations (Kenyon *et al*. 2000; Gardner 2001; Stadnitskaia *et al*. 2006; Hensen *et al*. 2007; Niemann *et al*. 2007).

In the shallow Moroccan margin the El Arraiche field encompasses Renard Ridge (including Pen Duick Escarpment), Vernadsky Ridge and several mud volcanoes (e.g. Al Idrisi, Mercator, Fíuza, Gemini, Kidd) located at depths from 200 to approximately 600m depth: The proximity to the euphotic zone and to the African coast adds to the great productivity observed in the area. Dead cold-water scleractinean coral reefs, carbonate crusts and exposed carbonate chimneys characterise the Renard and Vernadsky Ridges. Carbonate crusts, rock blocks and clasts are often found in the craters of the shallow mud

volcanoes where mild seepage activity has been recorded (Van Rensbergen *et al*. 2005). Mercator, one of the shallowest mud volcanoes differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water (Van Rensbergen *et al*. 2005). The top of Mercator shows patches of disturbed sediments from which gas venting is occasionally observed. Solitary corals (*Caryophyllia* sp.), accompanied by Cidaridae echinoids and Onuphidae polychaetes (*Hyalinoecia tubicola*) are the most conspicuous organisms seen during video surveys of the Mercator crater.

The western Moroccan field comprises several mud volcanoes (e.g. Meknès, Student, Yuma, Ginsburg, Jesuz Baraza, Darwin) at intermediate depths (700-1200m) located along an extensive province of carbonate and mostly dead cold water coral mounds. The widespread presence of authigenic carbonates and also extensive *Neptunea* and *Bathymodiolus* graveyards (usually within the crater of the mud volcanoes) suggest that this was a very active seepage area in the past. Darwin mud volcano differs from the others in this area because its crater is completely covered by large carbonate slabs and crusts; the fissures among slabs and depressions with scattered crust are filled with abundant shell ash and occasionally small clumps of living *Bathymodiolus mauritanicus* Meknès is the southernmost Moroccan mud volcano rising isolated among an extensive field of small coral mounds. The crater is formed by stiff, sometimes heavily disturbed, green mud breccia with scattered clasts and a striking large number of empty shells of the gastropod *Neptunea contraria*. Except for a few *Paromola cuvieri* individuals, living megafauna is rarely sighted in the crater.

The deep-water field (1300-4000m), mostly within the Portuguese margin includes several mud volcanoes (e.g. Captain Arutyunov, Carlos Ribeiro, Bonjardim. Porto) that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz (Kenyon *et al*. 2000, 2001, 2002, 2003, 2006; Akhmetzhanov *et al*. 2007, 2008). Video surveys of these deeper mud volcanoes often show conspicuous Siboglinid fields (e.g. Porto MV) in the active craters and exuberant sponge and

gorgonian patches at the crater rim and upper flank (e.g. Carlos Ribeiro mud volcano).

Sampling. Samples were collected between 2002 and 2006 during TTR12, TTR 14, TTR15 and TTR16 cruises onboard RV Prof. Logachev (MSU) and MSM.01-03 cruise onboard RV Maria S. Merian (IFM-GEOMAR). The material was collected using TV-assisted grabs or USNEL box-corers. Occasionally faunal specimens were also recovered from Kasten-corer, multiple corer or lander samples that were carried out for different purposes. Whenever possible the specimens were sorted onboard and preserved in 70 or 96% ethanol (the latter preserved for molecular analysis).

2.3.3.Systematics

Class Bivalvia Linnaeus, 1758

Order Solemyoida Dall, 1889

Superfamily Solemyoidea (Gray, 1840)

Family Solemyidae Gray, 1840

Genus *Petrasma* Dall, 1908

Type species. *Solemya borealis* Totten, 1834

Definition. As given by Taylor *et al*. 2009. Ligament wholly internal, supported by a buttress and lacking posterior or lateral extensions.

***Petrasma elarraichensis* sp. nov** (Figure 2.3.1, Figure 2.3.2)

Material examined. *Holotype*: one specimen, TTR14, stn AT528GR, El Arraiche field, Kidd MV, 35°25.304'N, 06°43.972'W, 489m, 03 August 2004.

Paratypes: ten specimens, same data as holotype; seven specimens, TTR15, stn AT569GR, El Arraiche field, Mercator MV, 35°17.917'N, 06°38.717'W, 358m, 25 July 2007.

Other material examined: eight juveniles specimens, same data as holotype; two specimens, TTR12, stn AT407GR, El Arraiche field, Pen Duick Escarpment, 35°17.695'N, 06°47.082'W, 560m, 15 July 2002; three specimens, TTR14, stn AT560B, El Arraiche field, Kidd MV, 35°25.306'N,

06°43.976'W, 498m, 8 August 2004; one specimen, TTR15, stn AT586GR, Western Moroccan field, Meknès MV, 34º59.146'N, 07º04.380'W, 701m, 28 July 2005; four specimens, TTR16, stn AT604GR, Western Moroccan field, Yuma MV, 35°25.820'N, 07°06.330'W, 1030m, 29 May 2006; two specimens, TTR16, stn AT607GR, Western Moroccan field, Ginsburg MV, 35°22.677'N, 07°04.979'W, 983m, 29 May 2006.

Measurements

	Station	Length mm	Height mm	Posterior Length mm
Holotype	AT528GR	33.8	14.1	9.8
Paratype	AT528GR	29.2	10.5	8.0
Paratype	AT528GR	25.6	10.0	6.2
Paratype	AT528GR	23.1	8.4	6.0
Paratype	AT528GR	14.7	5.3	3.6
Paratype	AT528GR	22.0	7.9	6.0
Paratype	AT528GR	11.6	4.7	2.5
Paratype	AT528GR	17.6	6.8	5.2

Description. *Shell* (Figure 2.3.1, Figure 2.3.2): to 35mm in length. Fragile. Equivalve. Inequilateral, beaks situated at 1/4 length of shell from posterior margin. Outline subcylindirical, compressed, length about 2.6 times height, slightly deeper towards the anterior, dorsal and ventral margins subparallel, anterior margin more broadly rounded than anterior, posterior dorsal margin projecting a little. Beaks indistinct, umbos sunken. Hinge teeth absent. Ligament primarily internal, supported by a prominent chondrophore that extends only slightly as a chondrophore ridge around the posterior adductor, lacking posterior and anterior extensions but a small roughly heart shaped area is present in front of the chondrophore and this is also visible externally just behind the beaks. Periostracum persistent and extending well beyond the shell margin, initially yellowish brown in colour but darkening with growth to a dark chestnut brown. Sculpture of weak radial ridges, 5-6 over the posterior and 10-12 over median and anterior. Adductor scars impressed, dorsal part of posterior scar angulate where bounded by chondrophore ridge, anterior adductor scar larger, spatulate in outline.

Anatomy (Figure 2.3.3): posterior siphonal opening surrounded by a series of papillae: A single large dorsal papilla (dp) lies above two smaller but still large papillae (dmp) on the dorsal margin of the opening, below these is a short smooth section (sa) followed by a series of papillae increasing in size towrds the ventral margin, there are 6 primary papillae (psp) on either side

and a single ventral median papillae, between these on the inner side are smaller papillae (ssp); a pair of subsiphonal ridges (ssr) are present below the siphonal crown.

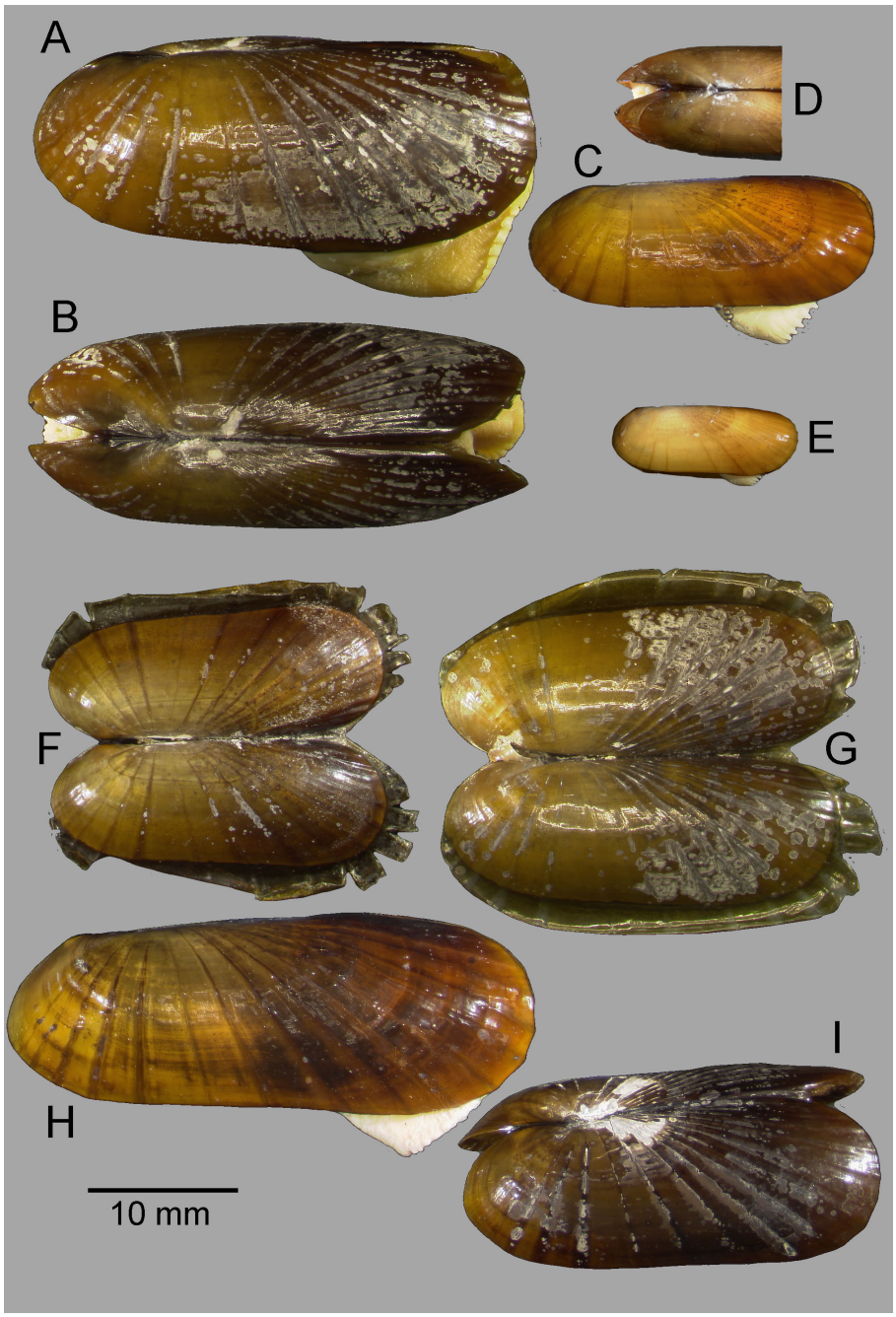

Figure 2.3.1. *Petrasma elarraichensis* sp. nov. A-E from Kidd MV, A-B lateral and dorsal views of holotype; C-D lateral and dorsal views of medium sized paratype; E lateral view of small paratype. F, paired valves from Pen Duick Escarpment; G, paired valves from Mercator MV; H lateral view of specimen from Meknès MV; I, lateral view of shell from Yuma MV.

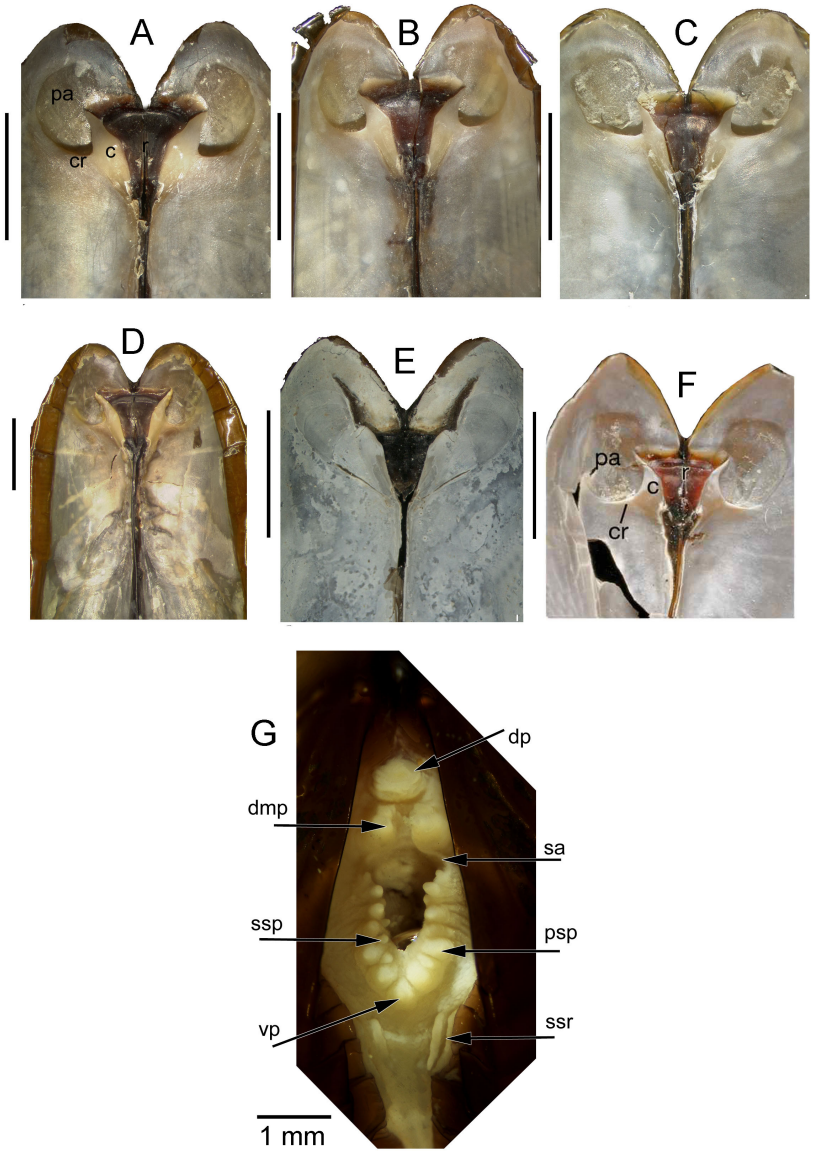

Figure 2.3.2. A-F, In ternal views of ligament, scale bars = 5mm. A-D, *Petrasma elarraichensis* sp. nov from A, Kidd MV; B, Pen Duick escarpement; C, Mercator MV; D, Yuma MV. E, *Solemya togata*, Mediterranean; F, *Petrasma velum*, Rhode Island (from Taylor *et al.* 2009). c, chondrophore; cr chondrophore ridge; pa, posterior adductor scar; r, resilium. G, posterior siphon of *P. elarraichensis*. dp, dorsal papilla; dmp, dorsal marginal papillae; psp, primary siphonal papillae; ssp, secondary siphonal papillae; ssr, subsiphonal ridge; vp, ventral papilla.

The mantle edge is fused from the posterior siphon for half the length of the ventral margin where there is a large anterior pedal gape. The mantle edge surrounding the rear of the foot bears a few tabulate papillae (pgp). The anterior dorsal mantle edge is prominently papillate (dap) and there is a single

papilla on the junction of the mantle edge anterior of the anterior adductor muscle (admp). The foot is very large with a broad oval sole, this fringed by large papillae, all equal in size. The ctenidium is large with numerous laminar filaments attached to a prominent gill axis. The palps are short twisted flattened with cup shaped terminations. The gut is present but difficult to examine due to its small dimensions but the hind gut and rectum were easily visible.

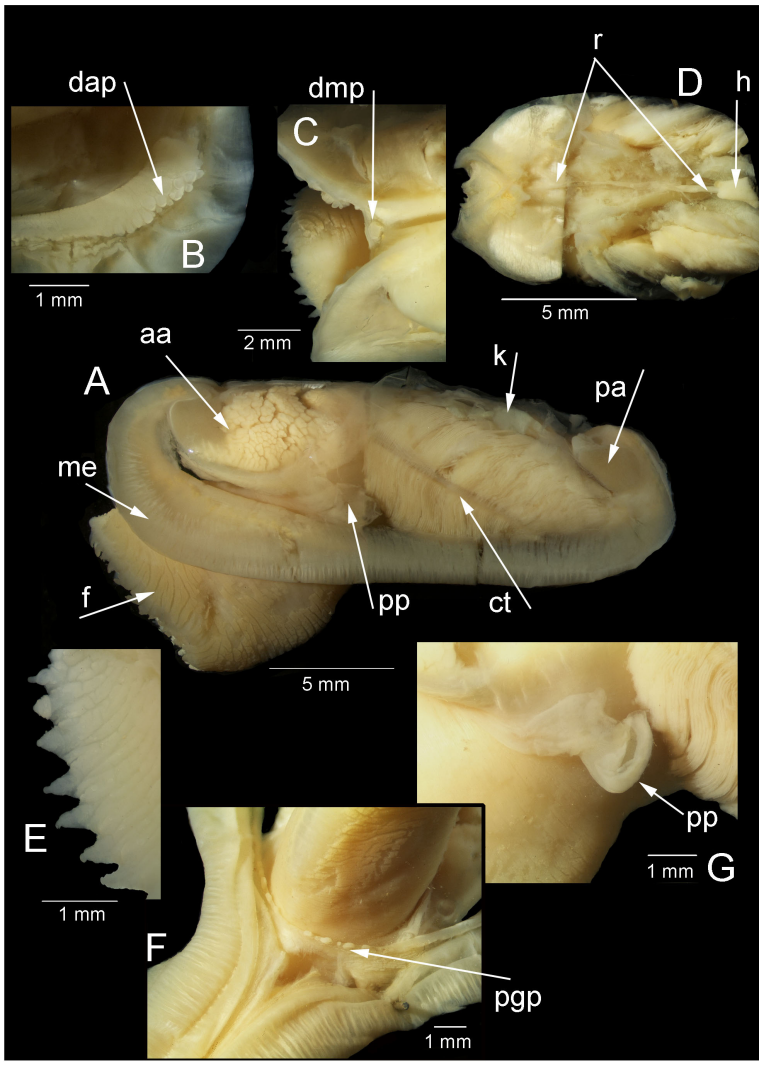

Figure 2.3.3. *Petrasma elarraichensis* sp. nov, Pen Duick, st. AT407GR, 560m. Anatomy. A, whole animal viewed from left side; B, papillae on dorsal anterior mantle edge; C, single, large papilla in dorsal median position; D, posterior dorsal dissection showing rectum passing through heart; E, marginal papillae on foot; F, papillae on mantle edge surrounding pedal gape; G, palp. aa, anterior adductor muscle. ct, ctenidium. dap, dorsal anterior papillae. dmp, dorsal median papilla. f, foot. h, heart. k, kidney. me, mantle edge. pa, posterior adductor muscle. pgp, papillae surrounding pedal gape. pp, palp. r, rectum.

Distribution. *Petrasma elarraichensis* is presently only known from the mud volcano fields in the Gulf of Cadiz, Eastern Atlantic. The majority of specimens have been taken from the El Arraiche field off the coast of Morocco in Kidd, Fiúza and Mercator mud volcanoes and the Pen Duick Escarpment at depths between 358-560m. A few specimens have been taken from the Western Moroccan field at the Meknès, Yuma, Ginsburg and Darwin mud volcanoes at the slightly deeper range of 700-1115m.

Etymology. elarraichensis, because of the majority of specimens have been taken from the El Arraiche field.

Remarks. The form of the ligament, which is primarily internal, supported by a chondrophore and lacks any lateral or anterior extensions, confirms the placement of *P. elarraichensis* in the genus *Petrasma* Dall, 1908 (Taylor *et al*. 2009). Some of the specimens carry an initial identification of "cf. *Solemya togata*" as might be expected from the proximity to the Mediterranean but the ligament of *Solemya* has prominent anterior extensions.

The genus *Petrasma* is not known from the North-East Atlantic but is represented in the Western Atlantic by a number of species. The most frequently encountered is *P. velum* (Say, 1822), from the north-east coast of America. Morphologically all species are very similar but we note that the curvature of the chondrophore and chondrophore ridge is circular in *P. velum* but angular in *P. elarraichensis*. Ecologically, however, the two species are very different with *P. velum* living in anaerobic sands and mud in sublittoral and shallow water. Mikkelsen & Bieler (2008) give a similar habitat for *P. occidentalis* (Deshayes, 1857) noting its occurrence in mangrove channels and around sewage outfalls. Abbott (1974) and Mikkelsen & Bieler (2008) note that *P. occidentalis* lacks any perceptable chondrophore ridge and give this as the main characteristic separating *P. occidentalis* from *P. velum*. The only other species of *Petrasma* in the North Atlantic is *P. borealis* Totten, 1834, which is found off the coast of north east USA and Canada in shallow water but further offshore than *P. velum* (Abbott, 1974, Conway *et a*l. 1992). Abbott (1974) notes that the siphon of *P. borealis* differs markedly from that of *P. velum* in having three pairs of long tentacles above the opening and a fringe of 15 pairs of shorter tentacles and papillae surrounding the lower part of the

opening. The siphon of *P. elarraichensis* is more similar to that of *P. velum* than to *P. borealis*. Conway *et al*. (1992) also consider *P. borealis* to lack a gut and this is in contrast with *P. velum* and *P. elarraichensis*.

Other North Atlantic species referred to as *Solemya*, *S. grandis* Verrill & Bush, 1898 and *S. caribbaea* Vokes, 1970 are excluded here because both belong to the genus *Acharax* (Abbott 1974).

In conclusion there are sufficient morphological and ecological grounds for considering the Gulf of Cadiz species of *Petrasma* to be new to science.

Genus *Acharax* Dall, 1908

Type species: *Solemya johnsoni* Dall, 1891

Definition. As given by Taylor *et al.* 2009. Ligament external, as a high arched band.

Acharax gadirae sp. nov. (Figure 2.3.4)

Type material. *Holotype*: one specimen, TTR12, stn AT391GR, Western Moroccan field, Jesuz Baraza MV, 35°35.439'N, 07°12.264'W, 1105m, 09 July 2002.

Paratypes: one specimen, same data as holotype; one shell, TTR 12, stn AT392G, deep-water field, Captain Arutyunov MV, 35º39.658'N, 07º20.018'W, 1320m, 9 July 2002; one shell, TTR 16, stn AT607GR, Western Moroccan field, Ginsburg MV, 35°22.677'N, 07°04.979'W, 983m. 29 May 2006.

Other material examined: one specimen, TTR16, st n AT602GR, El Arraiche field, Pen Duick Escarpment, 35°17.693'N 06°47.089'W, 556m, 28 May 2006; one specimen, TTR16, stn AT604GR, Western Moroccan field, Yuma MV, 35°25.820'N, 07°06.330'W, 1030m, 29 May 2006; one specimen, TTR16, stn AT605GR, same locality, 35°25.046'N, 07°05.450'W, 975m, 29 May 2006; one specimen, TTR16, stn AT615GR, deep-water field, Carlos Ribeiro MV, 35°47.238'N, 08°25.272'W, 2200m, 31 May 2006; one specimen, TTR16, stn AT617K, same locality, 35º47.246'N, 08º25.303'W, 2230m, 31 May 2006; two specimens, MSM01.03, stn 145, deep-water field, Porto MV, 35°33.773'N, 09°30.416'W, 3902m, 3 June 2006.

Measurements

	Station	Calcified Shell Length mm	Calcified Shell Height mm	Calcified Shell Posterior Length mm	Actual length mm
Holotype	AT391GR	56.3	21.1	14.49	59.5
Paratype	AT391GR	60.0	22.0	14.20	65.4
Paratype	AT392GR	67.0	25.0	19.10	85.0
Paratype	AT607GR	42.8	15.9	10.60	48.9

Description (Figure 2.3.4) Calcified shell to 67mm in length, to 85mm including periostracal fringe. Robust. Equivalve. Inequilateral, beaks situated at 1/4 length of shell from posterior margin. Outline subcylindirical, compressed, calcified shell length about 3 times height, slightly deeper towards the anterior, dorsal and ventral margins subparallel, anterior margin more broadly rounded than anterior, posterior dorsal margin projecting a little. Including periostracal fringe, anterior appears greatly expanded compared with posterior. Beaks indistinct, umbos sunken. Hinge teeth absent. Ligament external, as a high arched band posterior of the beaks and supported by a thickened shell margin; an oval area of ligament is present immediately behind the beaks and visible internally, anterior of the beaks shell margins fused by periostracal material along entire dorsal margins. Periostracum persistent and extending well beyond the shell margin, initially yellowish brown in colour but darkening with growth to dark brown and black; periostracal frill thickened over ribs but entire. Sculpture of radial ridges, 4-5 closely spaced over the posterior; median area almost smooth with 2-3 low ribs; posterior with 7-9 deeply cut ribs. Adductor scars impressed, posterior scar subcircular, anterior adductor scar larger, spatulate in outline. Anterior inner shell margin scalloped corresponding to radial ribs.

Posterior siphonal opening surrounded by a series of papillae:- A single large dorsal papilla (dp) lies above 2-3 pairs of slightly smaller papillae (dmp) on the dorsal margin of the opening, below these surrounding the opening is a series of approximately alternating large and small papillae with those most ventral the largest.

The mantle edge is fused from the posterior siphon for half the length of the ventral margin where there is a large anterior pedal gape. The mantle edge surrounding the rear of the foot is papillate (pgp). The anterior dorsal mantle edge is prominently papillate (dap). The foot is very large with a broad

oval sole, the margin interdigitates between large and small blunt papillae. The ctenidium is large with numerous laminar filaments attached to a prominent gill axis. The palps are short twisted flattened with cup shaped terminations. The presence or absence of a gut could not be confirmed.

Distribution. *Acharax gadirae* is presently only known from the mud volcano fields in the Gulf of Cadiz, Eastern Atlantic. The specimens have been taken from the Western Moroccan field at Yuma, Ginsburg and Jesuz Baraza mud volcanoes, and from the deep-water field at Captain Arutyunov, Carlos Ribeiro and Porto mud volcanoes at depths between 975 to 3902m. A single specimen was recovered from the shallower El Arraiche field in Pen Duick Escarpment at 556m.

Etymology. gadirae, from the Phoenician "Gadir" the original name for Cadiz and meaning "walled fortification" and also the root of many Moroccan names such as Agadir. Named to indicate the widespread range across the Moroccan and Iberian margins.

Remarks. The genus *Acharax* is recognisable from the large external ligament and the generic placement of *A. gadirae* is confirmed.

The genus is rare in the Atlantic Ocean unlike the situation in the Pacific where species of *Acharax* are frequently recorded from chemosynthetic settings (Neulinger *et al.* 2006). Only two species are known from the Atlantic. *Acharax grandis* (Verrill & Bush 1898) is known only from the original material collected from depths between 480 and 2700m in the region of the New York Bight. *Acharax caribbaea* (Vokes, 1970), again is only recorded from the original material collected from a depth of 350m off Colombia in the Caribbean Sea. However the genus is recorded in recent studies from both the Gulf of Mexico (Carney 1994) and the Barbados prism (Olu *et al.* 1997) but the species are not identified.

Acharax grandis differs from both *A. gadirae* and *A. caribbaea* in being less inequilateral with the beaks distinctly more towards the mid-line. *Acharax caribbaea* differs from both *A. gadirae* and A. grandis in having very few (4) anterior ribs compared with the 6-8 on *A. grandis* and 10-12 on *A. gadirae*.

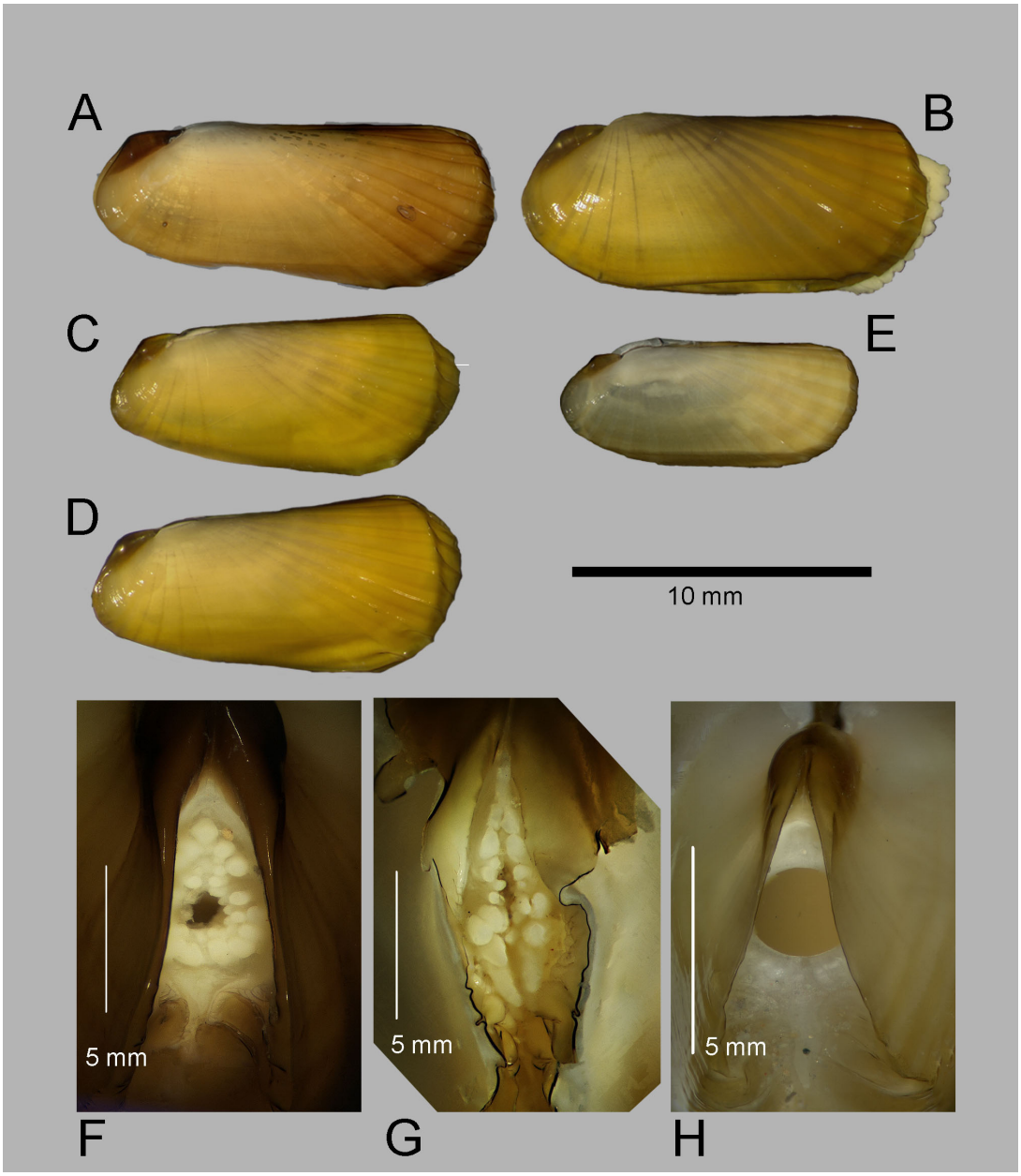

Figure 2.3.4. A-F *Acharax gadirae* sp. nov. A, Holotype, st. AT391GR, Jesuz Baraza MV; B, Paratype, st. AT607GR, Ginsburg MV; C, interior view of posterior ligament, st. AT392GR, Jesuz Baraza MV; D, anterior dorsal mantle edge, st. AT391GR; E, posterior siphon, st AT391GR; F, foot, st. AT391GR

There are no given ecological data for either *A. grandis* or *A. caribbaea*. The type locality for *A. grandis*, which is the region around the Hudson Shelf and Canyon, has no recorded seep or vent activity. In contrast the region around the type locality of *A. caribbaea* is known for a variety of chemosynthetic settings (Carney 1994)

The bathymetric range of *Acharax* in the Gulf of Cadiz is large, 556-3902m and specimens have been taken at many mud volcanoes raising the possibility that more than one species is involved. Unfortunately the specimens from the abyssal sites are all small about 10mm or less making comparison with the large specimens from the bathyal sites inconclusive. The specimens from Carlos Ribeiro (2200m) are prominently wedge shaped in outline compared with the specimen from Porto mud volcano (3902m). The latter is not dissimilar to those from Capt. Arutyunov (1325m) with the specimen from Pen Duick (556m) somewhat more elongate but not as wedge shaped as those from Carlos Ribeiro mud volcano.

The specimens from the Capt Arutyunov mud volcano are most problematic in that the siphonal opening appears to be devoid of any surrounding tentacles or papillae. This does not appear to be a function of size as similar specimens from other sites have siphonal papillae. Should this observation be confirmed in further material it would be appropriate to describe this as a separate species. Comparing the siphonal papillae of specimens from Pen Duick and Carlos Ribeiro indicates a more complex arrangement in the latter but, with so few specimens, this is inconclusive.

Superfamily Lucinoidea Fleming, 1828

Family Lucinidae Fleming, 1828

Genus *Lucinoma* Dall, 1901

Type species. *Lucina filosa* Stimpson, 1851
Definition. As given by Oliver & Holmes (2006)

Lucinoma asapheus sp. nov. (Figure 2.3.5)

Type material. *Holotype*; one complete specimens, live collected, TTR 15, stn AT569GR, El Arraiche field, Mercator MV. 35º17.917'N, 06º38.717'W, 358m, 25 July 2005.

Paratypes; Five specimens, same data as holotype.

Measurements

	Length	Height	Width	Ant. scar length	Ant scar angle	Lunule width
Holotype	33.3	30.1	15.9	15.0	15	2.2
Paratype	25.0	23.0	10.8	11.3	17	1.6
Paratype	30.7	27.6	18.0	14.6	16	2.6
Paratype	28.8	27.1	13.5	13.0	15	1.7
Paratype	32.1	29.2	16.4	16.4	18	2.6
Paratype	31.7	29.4	15.4	16.4	15	2.2

Description (Figure 2.3.5). Shell to 34 mm in length. Solid. Equivalve. Equilateral. Tumidity variable (Figure 2.3.5: G&H) but mostly rather compressed. Umbos low, beaks pointing forward. Outline lenticular; posterior dorsal margin almost straight, sloping gently; posterior margin curved but less so than anterior; anterior dorsal margin short, a little concave. Escutcheon narrow, edges slightly raised, extending the length of the posterior dorsal margin; three-quarters filled by ligament, remainder smooth. Lunule distinct, width dependant on tumidity of shell; edges raised, sharp. Sculpture of numerous, low but erect, thin, concentric lamellae; btween lamellae are weak concentric lines. Ligament external as a prominent raised arched band. Set on a narrow nymph. Hinge weak; two small cardinal teeth in each valve, RV anterior and LV posterior weakly bifid; anterior lateral protuberance distinct to obscure. Pallial line entire. Anterior adductor scar greatly elongate, approximately 3/4 free from pallial line. Shell white, periostracum thin but persistent, straw coloured (all material collected has been stained in Rose Bengal, thus the pink tinge).

The anatomy is essentially that described for *L. borealis* by Allen (1958) and for *L. kazani* by Salas & Woodside (2002). The inhalant siphon is surrounded by numerous short tentacles and papillae (Figure 2.3.5: I)

Variation. The shell can be rather compressed (Figure 2.3.5: H) or tumid (Figure 2.3.5: G) and this may be related to age rather than size as suggested by Oliver & Holmes (2006a) for *L. gagei* from the Arabian Sea. Some shells also show distortion with radial depressions developing abruptly (Figure 2.3.5: E).

Molecular data. Tissues were sent to Dr. John Taylor (NHM, London) for inclusion in his survey of Lucinidae and the 16S and CO1 genes were

compared with those of *Lucinoma borealis*. The results indicate that the two populations are not conspecific.

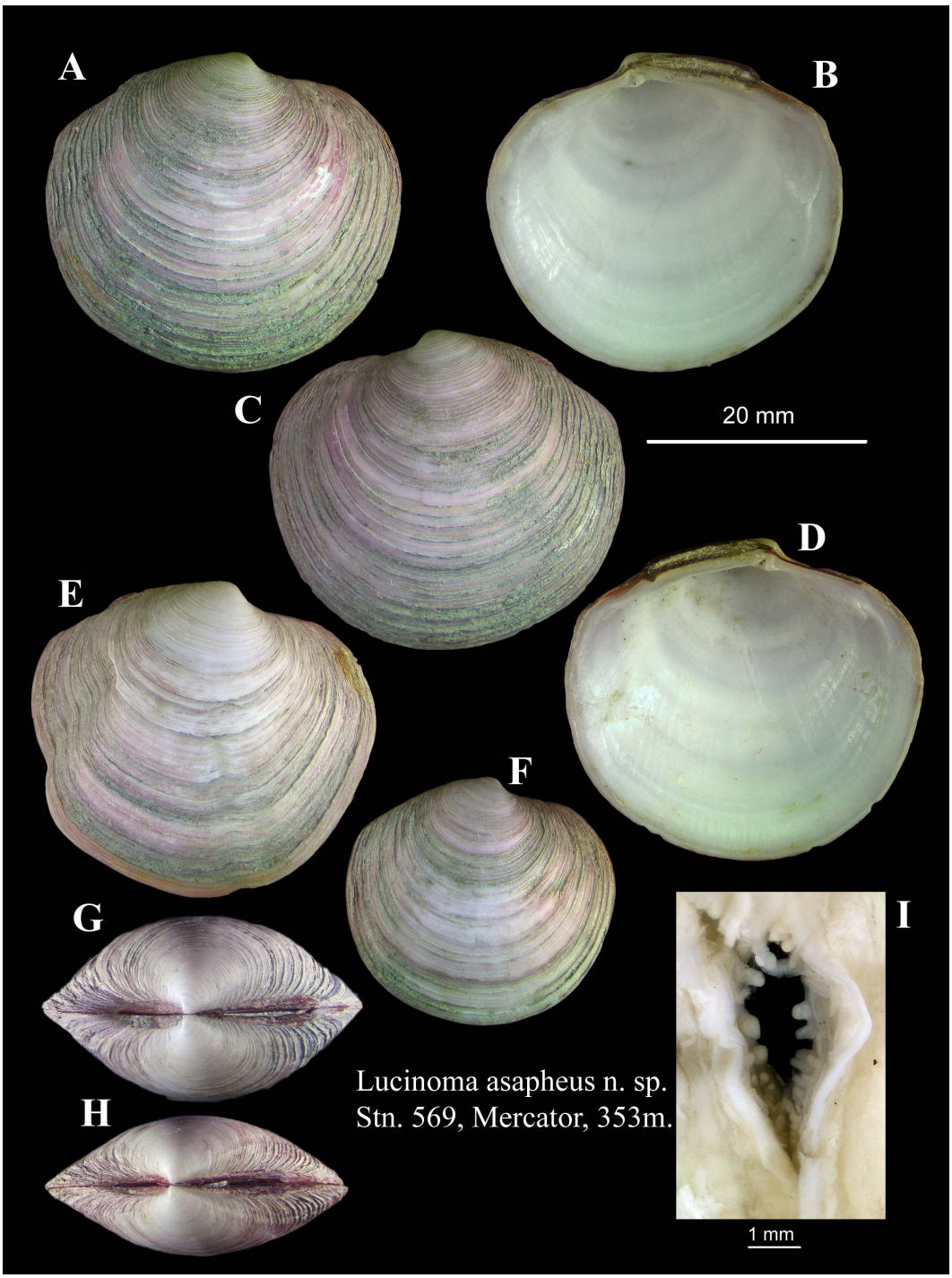

Figure 2.3.5. *Lucinoma asapheus* sp. nov. st. AT569GR, Mercator MV. A-D, Holotype, NMW.Z. ?????. E, an aberrant specimen. F, a small specimen. G-H two specimens showing variation in tumidity. I, the inhalant siphon.

Distribution. Only found live at Mercator mud volcano in the Gulf of Cadiz (358m).

Etymology. asapheus from asaphes Greek: meaning "indistinct" and "baffling", referring to the lack of distinctive morphological characters and the consequent unsettling taxonomic issues.

Remarks. A morphometric analysis was done comparing the Gulf of Cadiz shells with those of *L. borealis* from numerous localities from around the British Isles. This analysis could not demonstrate any statistically valid differences in the outline, the relative size of the anterior adductor scar or the angle of divergence of this scar from the pallial line. Anatomically *L. asapheus* and *L. borealis* are alike including the papillae that surround the inhalant aperture. The only conclusive evidence for the species level distinction between *L. asapheus* and *L. borealis* is that from the molecular data. Ecologically one might expect mud volcanoes and near shore sulphide enriched sediments to support different species. This argument was used by Salas & Woodside (2002) to support the distinction between *L. kazani* and *L. borealis*, but they also listed some morphological differences. Some of these, namely the tumidity of the valves, the width of the lunule and the expression of the lateral teeth are found here to be variable and therefore not conclusive. The papillation of the inhalant siphon does appear to be much less developed in *L. kazani* compared with that in *L. borealis* and *L. asapheus*. The angle of divergence of the anterior adductor scar also shows a difference with that in *L.kazani* having a mean value of ca. 25° and both *L. borealis* and *L. asapheus* a mean value of ca. 15°. Given that *L. kazani* and *L. asapheus* both inhabit mud volcano settings one might expect them to be conspecific. However, accepting the morphological differences given by Salas & Woodside between *L. kazani* and *L. borealis* and that these also hold true for *L. asapheus* then the two must be considered distinct. This may be supported by the wide difference in depth range with *L. asapheus* coming from 358m in contrast to *L. kazani* from 1700 –2030m.

Family Thyasiridae Dall, 1901

Genus *Spinaxinus* Oliver & Holmes, 2006

Type species. *Spinaxinus sentosus* Oliver & Holmes, 2006
Definition. As given by Oliver & Holmes (2006)

Spinaxinus cf. sentosus Oliver & Holmes, 2006 (Figure 2.3.6)

Material examined. one live collected specimen, MSM01.03, Stn 190, deep-water field, Captain Arutyunov MV, 35º39.665'N, 07º19.970'W, 1322m, 28 April 2006.

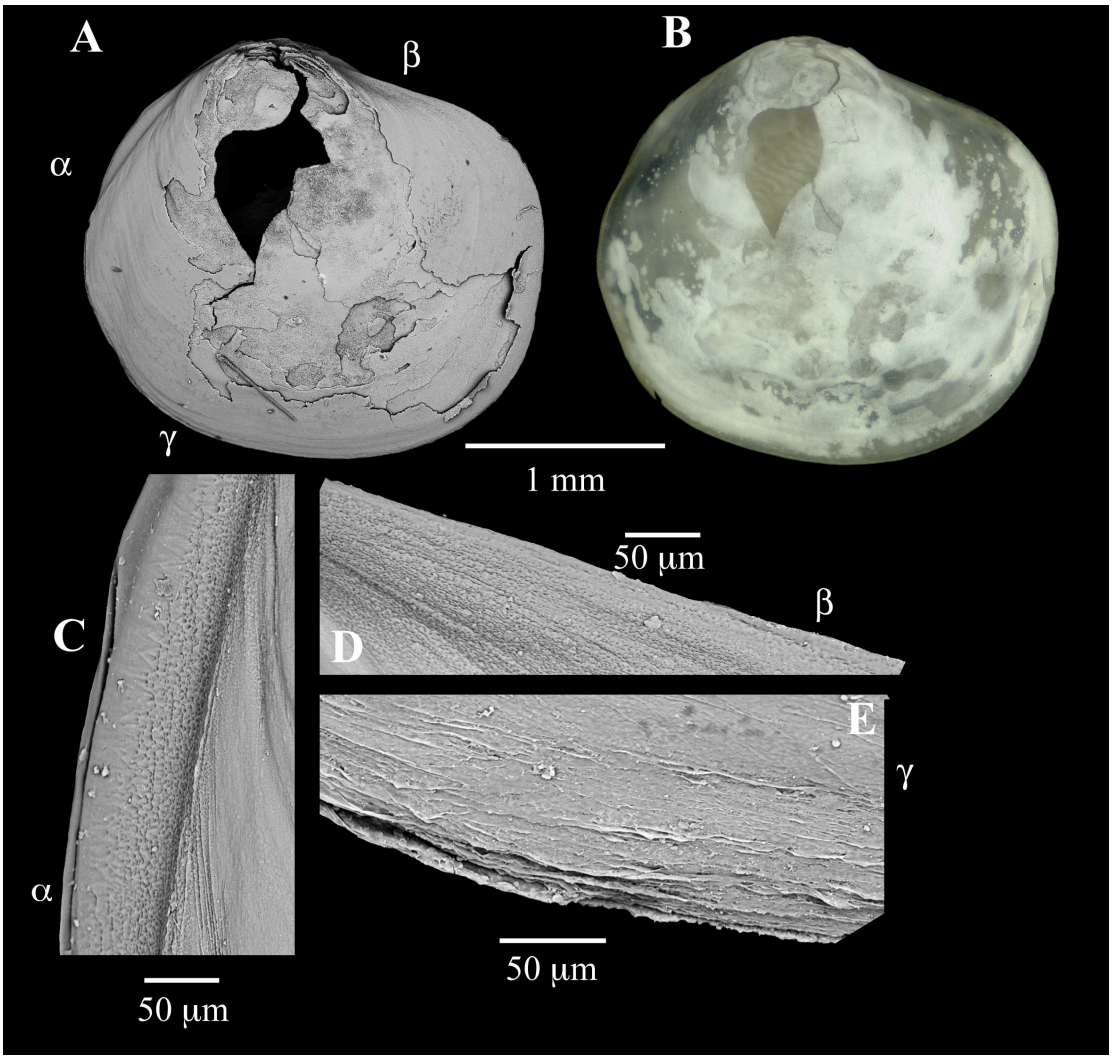

Figure 2.3.6. *Spinaxinus sentosus* Oliver & Holmes 2006. st. 190-MUC-08, Captain Arutyunov MV. A, SEM of right valve. B, digital image of right valve. C, periostracum on posterior margin. D, periostracum on anterior dorsal margin. E, periostracum on ventral margin.

Description (Figure 2.3.6). This specimen measures only 2.3 mm in length and is damaged. The outline agrees with that of *S. sentosus* in being extended anteriorly with a long lunule depression and in the presence of a long but shallow posterior sulcus. These features are in contrast to the juveniles of *Thyasira vulcolutre* Rodrigues & Oliver (2008), which also occurs at similar depths and settings.

The periostracal spines are typical of *Spinaxinus* but are not seen in this specimen. The periostracum is coarse and the vestiges of lamellae and projections can be seen on the extreme edges of the shell especially on the ventral margin.

Although the identification is not conclusive the likelihood that this shell is a juvenile *Spinaxinus* is high and as such represents the first finding of this species in a non-anthropogenic setting. The proximity of the type locality (off northern Portugal) to the Cadiz mud volcanoes makes this supposition more reasonable.

Superfamily Glossoidea Gray, 1847

Family Vesicomyidae Dall & Simpson, 1901

Genus *Isorropodon* Sturany, 1896

Type species*. Isorropodon perplexum* Sturany, 1896

Definition. As given by Cosel and Salas (2001)

Isorropodon megadesmus sp. nov (Figure 2.3.7; Figure 2.3.8: C-D)

Material examined. *Holotype*: one complete specimen, live collected, MSM01.03, stn 218, deep-water field, Captain Arutyunov MV. 35º39.642'N, 07º20.049'W, 1321m, 30 April 2006.

Paratypes: ten specimens, four shells and one valve, same data as holotype.

Other material examined: over thirty decalcified juvenile specimens, MSM01.03, stn 218, deep-water field, Captain Arutyunov MV. 35º39.642'N, 07º20.049'W, 1321m, 30 April 2006; one specimen, MSM01.03, stn 225, same locality. 35º39.707'N, 07º20.020'W, 1322m, 4 May 2006.

Measurements

	Length	Height	Tumidity	Ratio (L/T)
Holotype	11.2	8.2	2.3 (4.6)	2.4
Paratype	14.8	11.5	3.25 (6.5)	2.3
Paratype	10.6	7.7	2.3 (4.6)	2.3
Paratype	6.2	4.4	1.3 (2.6)	
Paratype	6.6	4.5	1.3 (2.6)	
Paratype	9.1	6.4	1.85 (3.7)	2.5
Paratype	9.6	7.2		

Description. (Figure 2.3.7; Figure 2.3.8: C-D). To 15mm in length. Thin. Equivalve. Inequilateral, beaks infront of the midline. Compressed, length to tumidity ratio 2.3 to 2.5. Outline subovate, anterior rounded, posterior a little obliquely truncated; ventral curvature at its maximum well to the posterior of the mid line. Lunule indistinct, not depressed. Escutcheon narrow, deeply excavated but entirely occupied by ligament. Sculpture of dense concentric lines and irregular growth stops or wrinkles. Hinge plate prominent dominated by a long nymph supporting a very large external ligament; ligament rises well above the dorsal margin of the shell and extends posteriorly beyond the nymph to fill the escutcheon. Hinge teeth complex; RV with a single prominent anterior lateral tooth situated in front of the beak in the form of a narrow projecting peg with a flat or slightly excavated dorsal surface; below the beak is an arched laminar tooth its anterior end overlapping the lateral tooth, its posterior slopes steeply and ventrally and merges with a second ridge only noticeable by a weak notch mid way on this combined ridge. LV with a thin laminar posterior cardinal angled obliquely plus two combined cardinals in a horizontal orientation the posterior part larger than the anterior with a distinct notch between the two parts. Pallial line entire with a very small straightened section below the posterior adductor scar; adductor scars of about equal size; anterior pedal retractor scar deeply impressed, situated immediately in front of the hinge plate. Periostracum thin, persistent, glossy. Shell white.

Mantle thin, mantle edge unfused except for short inhalant and exhalant siphonal apertures; inhalant aperture with many papillae increasing in size dorsally, exhalant with papillae of equal size. Foot with a distinct finger-like toe and poorly developed heel, pedal retractors prominent, the anterior attached in a deep impression close to the hinge. Anterior adductor muscle oval in cross-section, posterior adductor muscle subcircular, smaller than the anterior one. Ctenidia of a large, single (inner) demibranch, ascending part

approximately one half the height of the outer, filaments fine tightly connected.

Distribution. *Isorropodon megadesmus* is restricted to Captain Arutyunov mud volcano (1321-1322m).

Etymology. megadesmus from the greek *mega* meaning large and *desma* meaning bond; referring to the external ligament.

Remarks. The taxonomy of *Isorropodon* in the Atlantic and Mediterranean was already seen to be complex by Cosel & Salas (2001). They stated that *Isorropodon* species are variable with regard to outline, tumidity and development of hinge teeth and this is illustrated in their figures 36-47 for *I. perplexum*. They noted similarities in shell morphology between the Eastern Mediterranean species *I. perplexum* and the West African *I. bigoti* but suggested that these taxa were isolated geographically and doubtfully could have gene flow between them. They further supported this argument by stating that *I. perplexum* had not been found in the Western Mediterranean or Ibero-Moroccan Gulf. With the discovery of *Isorropodon* at the Capt. Arutyunov mud volcano such a theory of isolation must be questioned and the taxonomy must be based on morphological characters.

In contrast to the variability given by Salas & Cosel for *I. perplexum* all of the shells from station 218 examined here are constant with regard to features of outline, tumidity and hinge teeth. However the single shell from station 180 is distinct being inflated, having a distinct lunule, having a much smaller ligament and in the ventral margin being more convex.

The shells from station 218 are distinct from all the shells of *I. perplexum* figured by Cosel & Salas (2001) in having a much longer nymph with the posterior teeth reaching only about one third of the ligament as opposed to the half distance given for *I. perplexum* by Cosel & Salas (2001). Furthmore, most of the shells illustrated by them have a more convex ventral margin than the shells from station 218. The single shell from station 180 shares more features with the Mediterranean shells in having the small ligament, convex ventral margin and being more inflated. The outline of *O. bigoti* differs from all of the above in the narrower anterior and distinct angulation of the ventral

curve, but is has a short nymph similar to *I. perplexum* and the shell from station 180.

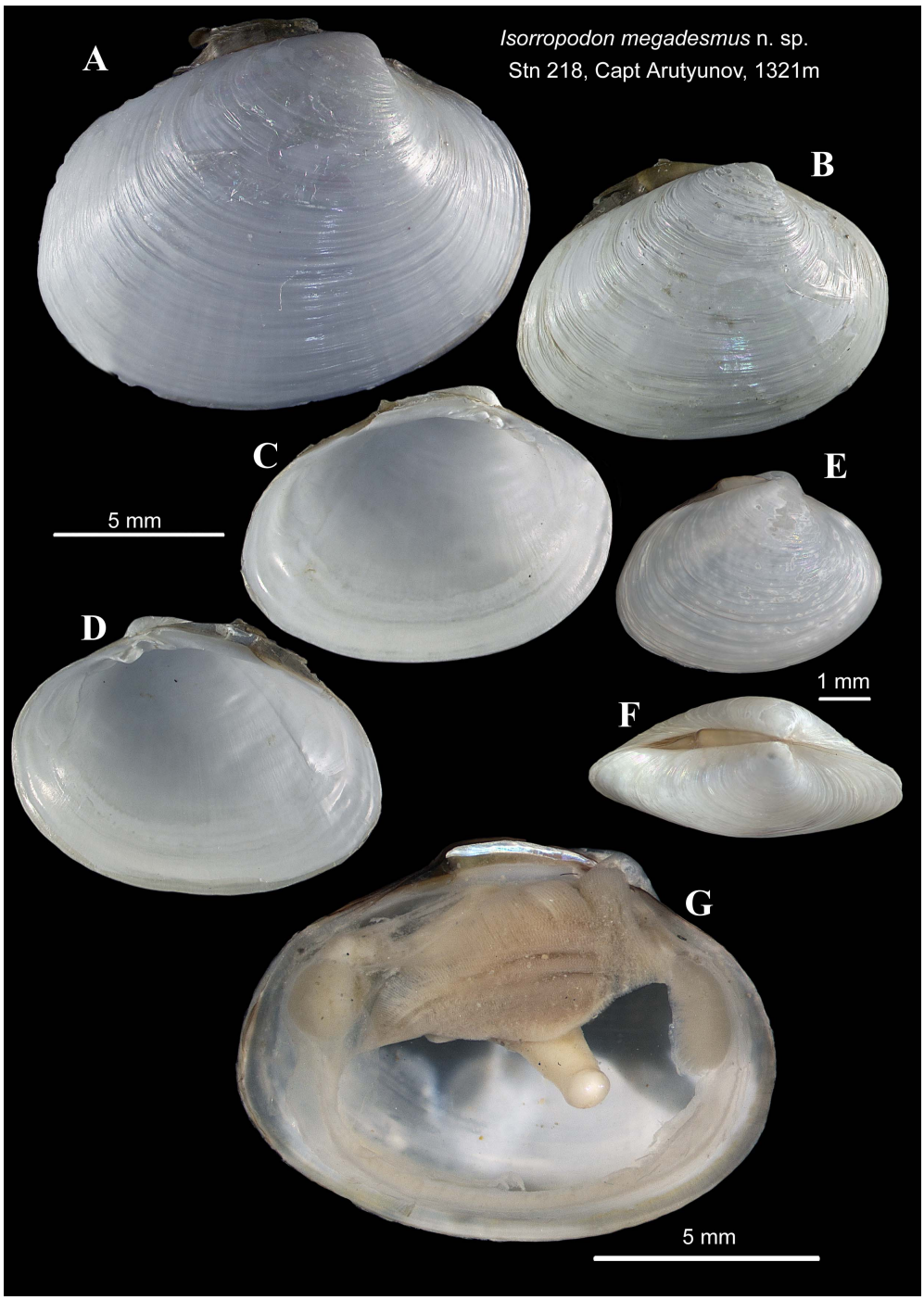

Figure 2.3.7. *Isorropodon megadesmus* sp. nov st. 218-GKG10, Captain Arutyunov MV. A, large right valve, paratype. B-D, right valve external, left valve internal, right valve internal, holotype. E, right valve small right valve, paratype. F, dorsal view, paratype. G, gross anatomy viewed after removal of right valve and mantle.

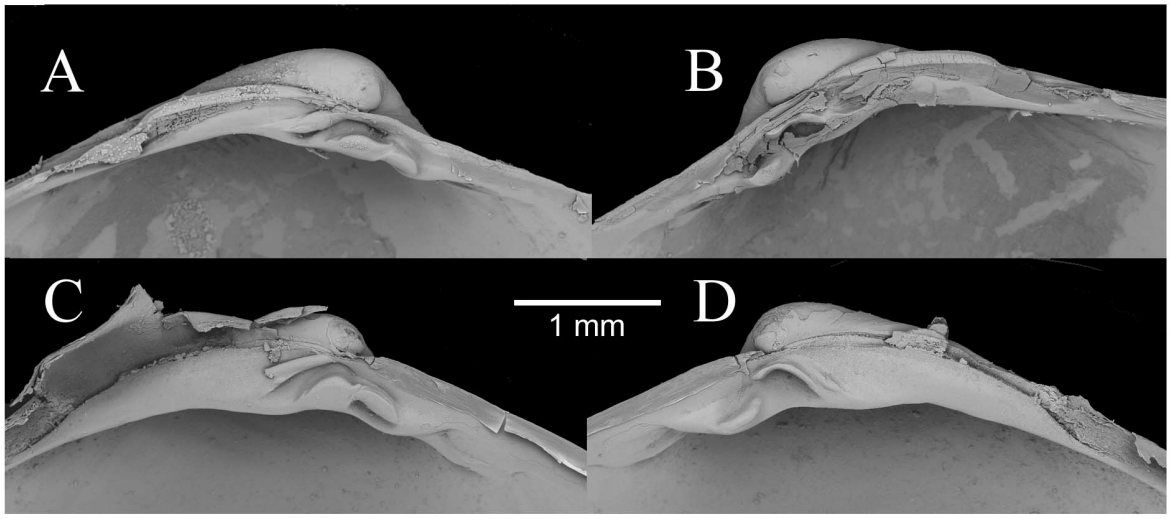

Figure 2.3.8. Comparision between the hinge teeth of *Isorropodon cf. perplexum* (A, B) and *Isorropodon megadesmus* sp. nov (C, D)

Following the study by Cosel & Salas one could be led to regard the Eastern Mediterranean, Gulf of Cadiz and West African material as conspecific. This could be further supported in that the shells from the Gulf of Cadiz come from the same mud volcano. However, we regard the constancy of the features of the shells from station 218 and the differences, especially the ligament to warrant separation from both *I. perplexum* and *I. bigoti.* We also regard the shells from station 218 and 180 to represent different species despite the fact that they come from the same mud volcano. However, with only a single specimen from station 180 and the similarities between it and *I. perplexum* as defined by Cosel & Salas (2001) we cannot identify it with certainty and associate with *I. perplexum*.

***Isorropodon cf. perplexum* Sturany 1896** (Figure 2.3.8: A-C; Figure 2.3.9)

Material examined. one complete specimen, live collected, MSM01.03, stn 180, deep-water field, Captain Arutyunov MV. 35º39.740'N, 07º19.960'W, 1323m, 27 April 2006.

Measurements. 6.4 (L) x 5.3 (H) x 3.6 (T)

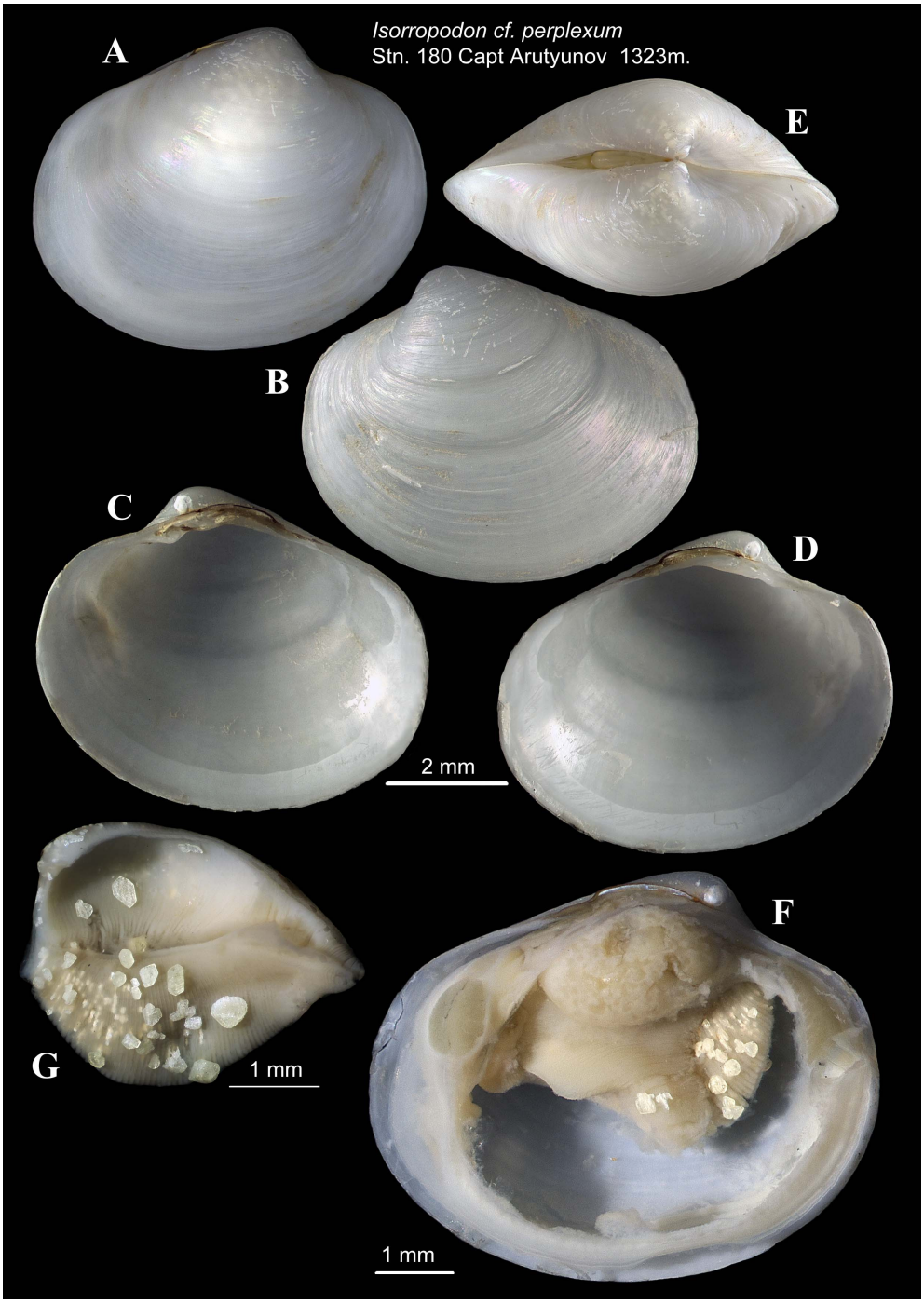

Figure 2.3.9. *Isorropodon cf. perplexum*, st 180 Captain Arutyunov MV. A, large right valve, B, left valve external; C-D, right valve external, left valve internal, right valve internal. E, dorsal view. F, gross anatomy viewed after removal of right valve and mantle. G, detail of gross anatomy.

Description (Figure 2.3.8: A-C; Figure 2.3.9). 6.4 mm in length. Thin. Equivalve. Inequilateral, beaks infront of the midline. Inflated, length to tumidity ratio = 1.8. Outline subovate, anterior bluntly rounded, posterior a

little obliquely truncated; ventral curvature at its maximum more or less at the midline. Lunule indistinct, slightly depressed. Escutcheon narrow, deeply excavated. Sculpture of dense, concentric, fine lines and few irregular growth stops. Hinge plate narrow, nymph supporting an external ligament; ligament scarcely rises above the dorsal margin of the shell and extends posteriorly to half the length of the escutcheon. Hinge teeth complex; RV with a single prominent anterior lateral tooth situated in front of the beak in the form of a narrow projecting peg with a flat or slightly excavated dorsal surface; below the beak is weakly arched laminar tooth its anterior end overlapping the lateral tooth, its posterior slopes steeply and ventrally and merges with a second ridge only noticeable by a weak notch mid way on this combined ridge. LV with a thin laminar posterior cardinal angled obliquely plus two combined cardinals in a horizontal orientation the posterior part only slightly larger than the anterior with a distinct notch between the two parts. Pallial line entire with a very small straightened section below the posterior adductor scar; adductor scars of about equal size; anterior pedal retractor scar deeply impressed, situated immediately in front of the hinge plate. Periostracum thin, persistent, glossy. Shell white

Mantle thin, mantle edge unfused except for short inhalant and exhalant siphonal apertures; inhalant aperture with few papillae increasing in size dorsally, the latter as short tentacles, exhalant with papillae of equal size. Foot with a blunt finger-like toe and poorly developed heel, pedal retractors prominent, the anterior attached in a deep impression close to the hinge. Anterior adductor muscle pyriform in cross-section, posterior adductor muscle subcircular, smaller than the anterior one. Ctenidia of a large, single (inner) demibranch, filling the majority of the mantle cavilty, ascending part approximately one half the height of the outer, filaments fine tightly connected.

The numerous crystalline growths seen on and between the filaments are believed to be natural and not an artifact of preservation.

Distribution. *Isorropodon cf. perplexum* is restricted to Captain Arutyunov mud volcano (1323m).

Remarks. As discussed above for *I. megadesmus*.

2.3.4.Brief Ecological Discussion

A total of ten bivalve species were found in close association with chemosynthetic settings in the Gulf of Cadiz (this paper, Génio *et al.* 2008; Rodrigues *et al.* 2008). The trophic status of these Solemyidae, Lucinidae, Thyasiridae and Vesicomyidae species have been confirmed by their global anatomic features (e.g. gills for thyasirids, reduced gut for solemyids) and, in some cases, also by stable isotope analysis (chapter 3.1) or endosymbiont DGGE analysis (Rodrigues *et al*. submitted, chapter 3.2). The $\delta^{13}C$ values for solemyid (*Acharax gadirae*, *Petrasma elarraichensis*), lucinid (*Lucinoma asapheus*) and thyasirid (*Thyasira vulcolutre*) bivalves were found to be in line with data for other bivalves known to host thiotrophic symbionts. On the other hand, $\delta^{13}C$ and $\delta^{34}S$ values for the bathymodiolid species (*Bathymodiolus mauritanicus*) were compatible with the predominance of methanotrophy. Results of bacterial 16S rRNA clone libraries demonstrated that all the species examined contained bacteria that were related to other known sulphur-oxidizing bacterial endosymbionts. The molecular results confirmed the thiotrophic nutrition for *P. elarraichensis*, *A. gadirae*, *L. asapheus* and *T. vulcolutre* but suggest a dual symbiosis for *B. mauritanicus* (Rodrigues *et al*. submitted). Nutrition of *Isorropodon megadesmus* was not yet confirmed by either isotopic or molecular analyses but other studies suggest that *Isorropodon perplexum* contain sulphur-oxidizing bacteria (Olu-Le Roy *et al*. 2004), a phylotype also present in *Spinaxinus sentosus* (Oliver & Holmes 2006)

Chemosymbiotic bivalves were more frequent in the shallower mud volcanoes but were especially diverse in Captain Arutyunov where five different species co-occur (Figure 2.3.10). The solemyid bivalves are the most common species of the chemosynthesis-based assemblage of the mud volcanoes from the Gulf of Cadiz. This is the first record this family in cold seeps from the North-east Atlantic, but several solemyid species have been found at cold seeps worlwide (Coan *et al*. 2000; Imhoff *et al*. 2003). The solemyids were represented by two species, *Petrasma* with a shallower distribution (358-1030m) and *Acharax* with a deeper distribution (556–

3902m) overlaping at intermediate depths in the Western Moroccan field where they were found co-occurring in some of the mud volcanoes.

Unlike *Petrasma*, *Acharax* species are consistently associated with seep or vent settings. This is also the first record of coexistence of both genera, however the morphological resemblance of the two genera can often lead to erroneous identifications which is likely to interfere with a correct knowledge of their present distributions. *Petrasma elarraichensis* seems to occur deeper than previously reported for *Solemya* spp. (Neulinger *et al*. 2006). Some *Acharax* specimens (from Yuma, Ginsburg and Jesuz Baraza mud volcanoes) host commensal polychates (Ravara *et al*. 2007).

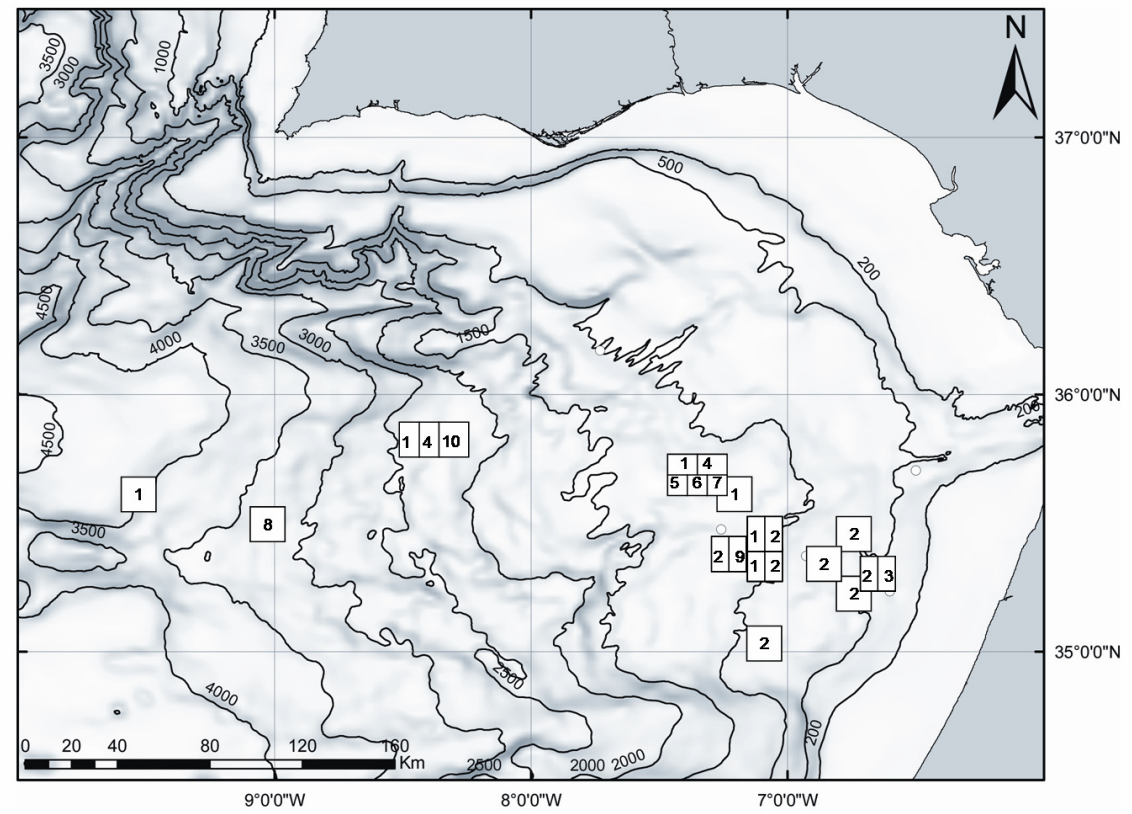

Figure 2.3.10. Mud volcanoes with chemosymbiotic species in the Gulf of Cadiz. 1, *Acharax gadirae*; 2, *Petrasma elairrachensis*; 3, *Lucinoma asapheus*; 4, *Thyasira vulcolutre*; 5, *Spinaxinus sentosus*; 6, *Isorropodon megadesmus*; 7, *Isorropodon* cf. *perplexum*; 8, *Calyptogena* cf *regab*; 9, *Bathymodiolus mauritanicus;* 10, *Idas* sp.

The lucinidae *Lucinoma asaphaeus* collected at Mercator mud volcano is very similar morphologically to *Lucinoma kazani* from the Eastern Mediterranean (Salas & Woodside 2002) and *Lucinoma borealis* abundant in the North-east Atlantic. Molecular analysis separated *Lucinoma borealis* and

species from the Gulf of Cadiz, but it was impossible until the moment test the other species. Nevertheless is likely that the mud volcano species may have evolved locally. Alive *Lucinoma* specimens were only collected in one mud volcano (Mercator) but video observations revealed the presence of this group in other mud volcanoes from the Spanish field (Cunha pers comm.). Another Lucinidae species *Myrtea amorpha* was been found at the Eastern Mediterranen mud volcanoes (Olu-Le Roy *et al*. 2004) and in the Alboran Sea (unpublished results).

The thyasirid *Thyasira vulcolutre* was only found in the deep-water mud volcano field (Rodrigues *et al*. 2008). Despite the high number of thyasiridae species present in the Gulf of Cadiz (eigth) only one *Thyasira vulcolutre* is strictly associated with active mud volcanoes (Rodrigues *et al*. 2008). *Spinaxinus sentosus* was discovered in the organic cargo of a sunken ship in the Atlantic Ocean (Oliver & Holmes 2006) and is reported here for the first time associated to a cold seep site. Other chemosymbiotic thyasirids (Table 2.3.1) were found associated with Siboglinidae fields from the HMMV (Pimenov *et al*. 2000; Gebruk *et al*. 2003) and from the eastern Mediterranean mud volcanoes (Olu-Le Roy *et al*. 2004).

Small Vesicomyids including *Isorropodon megadesmus* and *Isorropodon perplexum* were very abundant in Captain Arutyunov mud volcano. The species *I. perplexum* was previously known only from its type locality from Eastern Mediterranean (Table 2.3.1) and was referred to as having sulphur-oxidizing bacteria (Salas & Woodside 2002). During TTR17 a living specimen of *Calyptogena* was found for the first time in the Gulf of Cadiz. The specimen collected from Bonjardim has been identified as belonging to the species *Calyptogena regab* recently found in the Regab region (Krylova pers. comm.). Other large vesicomyid shells but no living specimens of *Laubericoncha chuni* and *Pliocardia* sp. were also collected at the same mud volcano. The vesicomyids *C. regab* and *L. chuni* dominate the faunal assemblages of Congo and Angola Basin cold seeps (Olu-Le Roy *et al*. 2007; Cosel & Olu 2008).

Table 2.3.1. Comparison between chemosymbiotic bivalve species from different cold seep sites. SOk: Sea of Okhotsk, JapT: Japan Trench; MB: Monterey Bay, GoM: Gulf of Mexico, Bar: Barbados, NM: Norwegian margin, GoC: Gulf of Cadiz; EM: Eastern Mediterranean, SEA: South East Atlantic. Data from Juniper & Sibuet (1987); Barry *et al*. (1998); Bergquist *et al*. (2003); Gebruk *et al*. (2003); Imhoff *et a*l. (2003); Olu *et al.* (1996); Olu *et al*. (1997); Olu-Le Roy *et al*. (2004); Olu-Le Roy *et al*. (2007); Cosel & Olu-Le Roy (2008).

	SoK	**JT**	**MB**	**GoM**	**Bar**	**NM**	**GoC**	**EM**	**SEA**
Solemyidae									
Acharax sp.	X								
Acharax gadirae							X		
Acharax johnsonii		X							
Petrasma elarraichensis							X		
Solemya sp.			X						
Lucinidae									
Lucinoma asapheus							X		
Lucinoma atlantis				X					
Lucinoma sp. nov.				X					
Lucinoma kasani								X	
Myrtea amorpha								X	
Thyasiridae									
Conchocele bisecta	X								
Maorithyas hadalis		X							
Spinaxinus sentosus							X		
Thyasira sp.						X			
Thyasira striata								X	
Thyasira vulcolutre							X		
Mytilidae									
Bathymodiolus sp.									X
Bathymodiolus boomerang					X				
Bathymodiolus childressi				X					
Bathymodiolus mauritanicus							X		
Bathymodiolus thermophilus					X				
Idas sp.							X		
Idas modiolaeformis								X	
Tamu fisheri				X					
Vesicomyidae						X			
Archivesica sp	X								
Calyptogena kilmeri			X						
Calyptogena aff. pacifica	X		X						
Calyptogena magnifica				X	X				
Calyptogena packardana			X						
Calyptogena phaseoliformis			X						
Calyptogena cf. *regab*							X		X
Calyptogena rectimargo	X								
Calyptogena soyoae		X							
Isorropodon megadesmus							X		
Isorropodon perplexum							X	X	

Bathymodiolin mussels of the amphi-Atlantic species *Bathymodiolus mauritanicus* were only found living in Darwin although extensive graveyards of mussel shell ash are also found in other mud volcanoes of the western Moroccan field (Génio *et al*. 2008). Although confirmed as chemosymbiotic, *Bathymodiolus mauritanicus* was first collected during a commercial trawl in the Mauritanian margin where no hydrocarbon seeps have yet been discovered. Nevertheless owing to the repeated appearance of other typical seep molluscs it is likely the existence of seepage in this region might be confirmed in the future (Cosel 2002). Another small mytilidae *Idas* sp. was collected living in a small wood fall collected with the ROV Isis at Carlos Ribeiro mud volcano during JC10. *Idas* specimens are present in the mud volcanoes from the Eastern Mediterranean (Table 2.3.1) and their symbioses have studied in detail by Duperron *et al*. (2008).

Of the 18 mud volcanoes sampled, 10 have chemosymbiotic species, which indicated their importance in the structure of the seep assemblages. Most of the ten chemosymbiotic species found in the Gulf of Cadiz are restricted to one or two mud volcanos and can result from physical or physiological constraints such as depth, distance and fluid flow rates.

The number of chemosymbiotic species is large when compared to other cold sites (table 2.3.1). Contrasting to the 10 species found in the Gulf of Cadiz, the other cold seeps present only two to six chemosymbiotic bivalve species. The diversity at genus level is also higher in the Gulf of Cadiz (9) in comparison with the other sites (4). In our opinion the diversity of chemosymbiotic bivalves in the Gulf of Cadiz is probably not completely revealed as frequent inhabitants of cold seeps, as the Vesicomyids (Table 2.3.1) were only observed during the most recent cruise in the deeper mud volcanoes.

The Gulf of Cadiz shares the bivalves *Bathymodiolus mauritanicus* and *Calyptogena regab* with the South East Atlantic off Africa, *Spinaxinus sentosus* from North Atlantic and *Isorropodon perplexum* with Eastern Mediterranean pointing out to the strategic position of Gulf of Cadiz for understanding the dispersion and evolution of cold seeps in the Mediterranean and Atlantic basins.

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Chapter 2.4.Biodiversity of bivalves (Mollusca: Bivalvia) from mud volcanoes and adjacent habitats in the Gulf of Cadiz (NE Atlantic)

Abstract

The deep-water bivalve assemblages were sampled from the mud volcanoes and adjacent habitats of the Gulf of Cadiz at bathyal depths from the shelf break to ~4000m. From the study of 759 specimens representing 49 species in 21 families, the diversity, distribution patterns and species turnover are described. Thyasiridae with eight species and Cuspidaridae with six species, were the best represented families by number of species but other small-sized, common deep-sea bivalves such as *Abra longicallus*, *Microgloma pusilla* and *Limopsis minuta* were the most frequent species followed by the seep-obligate Solemyidae (*Petrasma* sp. nov.; *Acharax* sp.nov.).

Keywords: Bivalvia, deep sea, mud volcanoes

Cunha MR, **Rodrigues CF** & Oliver PG (in preparation) Biodiversity of bivalves Mollusca: Bivalvia) from the mud volcanoes and adjacent habitats in the Gulf of Cadiz (NE Atlantic).

Biodiversity of bivalves (Mollusca: Bivalvia) from mud volcanoes and adjacent habitats in the Gulf of Cadiz (NE Atlantic)

2.4.1.Introduction

Bivalves account for 8% and 10% of the abundance in deep-sea macrofaunal assemblages being the third taxon in abundance after polychaetes and peracarid crustaceans (Gage & Tyler 1991). Bivalves are often reported from general studies on the deep-sea benthos zonation (e.g. Rex 1981; Rex *et al*. 1993, 1997, 2000; Grassle & Maciolek 1992). The most comprehensive study dedicated to the diversity and distributional patterns deep-sea bivalves encompasses ten abyssal basins in the Atlantic but focus only on the Protobranchia (Allen & Sanders 1996) and there are few areas where the deep-sea bivalve fauna has been well characterized (Linse 2004; Olabarria 2005).

Many aspects about the deep-sea environment and fauna seem to defy conventional theories on diversification and subsequent coexistence of species. There are few obvious barriers to gene flow, and a large proportion of species are now known to have pelagic larval dispersal (Young 2004). Local diversity can exceed 300 macrofaunal species m^2 (Etter & Mullineaux 2001), but most species exploit the same detrital food resources (Gage & Tyler 1991), and the structural habitat heterogeneity typical of other highly diverse systems such as coral reefs and kelp forests is generally lacking. The deep-water bivalve species are thought to be pre-adapted to life at depth through their ability to inhabit impoverished deoxygenated habitats. Few morphological differences could be detected between populations of species that occur in both shallow and deep water. The small size of all but a few species may be one of their greatest adaptative features (Payne & Allen 1991).

In areas of cold seeps the heterogeneity of habitat is gretly enhaced by geological and biogenic structures as well as by the peculiar biogeochemical conditions that sustain productive chemosynthesis-based assemblages (e.g. Sibuet & Olu 1998; Levin 2005; Cordes *et al.* in press). Contrasting with the

usual deep-sea assemblages in cold seeps the fauna is dominated by large-sized bivalves of five major bivalve families with typical symbiont-bearing: the Solemyidae, Lucinidae, Vesicomyidae, Thyasiridae and Mytilidae (Sibuet & Olu 1998; Sibuet & Olu-Le Roy 2002; Sahling *et al.* 2003; Levin 2005; Chapter 2.2 - Rodrigues *at al.* 2008; Chapter 2.3). The recent investigations carried out in the in the Gulf of Cadiz provided the opportunity to study the bivalve assemblages from mud volcanoes and adjacent habitats in this extensive seepage area. Although the bivalve fauna from the Southern Iberian Peninsula have already been described from samples taken during the Balgim and Fauna1 cruises (Salas 1996) the study areas seemed to have coincidentaly avoied the important mud volcanos, carbonate mounds and cold-water coral fields of the region. Herein the diversity, distributional patterns and species turnover of the bivalve assemblages associated to these environments are examined.

2.4.2.Material and methods

Environmental setting. The Gulf of Cadiz is located in the NE Atlantic Ocean between 34ºN and 37º15'N and 6ºW to 9º45'W. It is enclosed by the southern Iberian and northern Moroccan margins, west of Gibraltar Strait. The Atlantic Ocean at the latitude of the Gulf of Cadiz and off Iberia is characterized by four main water masses with different characteristics of temperature, salinity, oxygen content and current velocities (e.g. Emery & Meincke 1986; Ambar & Howe 1979; Ambar *et al*. 2002). These are the Eastern North Atlantic Central Water that may circulate in the Gulf of Cadiz down to 850m; the Mediterranean Water flows westwards along the Southern Iberian slope with an upper core between 500 and 800m and a lower core centred at about 1200m; the North Atlantic Deep Water flowing southward, bellow 2000m depth and the Antarctic Bottom Water that circulates at depths greater than 4000m and therefore does not have direct influence in the Gulf of Cadiz. The present day circulation patterns and the hydrographical conditions in the Gulf of Cadiz are dominated by the exchange of water masses through the Strait of

Gibraltar, the frequent generation of meddies and recirculation of offshore water (Peliz *et al*. 2006).

The geological history of the Gulf of Cadiz is intimately related to plate tectonic interaction between Southern Eurasia and North Africa and is driven by two major mechanisms: a) subduction associated with the westward emplacement of the Gibraltar Arc and formation of the Gulf of Cadiz accretionary wedge, probably not active at present and b) oblique lithosphere collision between Iberia and Nubia, active at present and causing active thrusting (Zitellini *et al*. 2009). It is now well established that the whole area is under compressive deformation and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages. This extensive area encompasses over forty mud volcanoes (confirmed by coring), at depths ranging from 200 to 4000m (Kenyon *et al*. 2000, 2001, 2002, 2003, 2006; Pinheiro *et al*. 2003; Akhmetzhanov *et al*. 2007, 2008), and active methane seepage has been documented on several locations (Kenyon *et al.* 2000; Gardner 2001; Stadnitskaia *et al*. 2006; Hensen *et al*. 2007; Niemann *et al*. 2007).

In the shallow Moroccan margin, the El Arraiche field encompasses Renard Ridge (including Pen Duick Escarpment), Vernadsky Ridge and several mud volcanoes (e.g. Al Idrisi, Mercator, Fíuza, Kidd) located at depths from 230 to approximately 600m depth: The proximity to the euphotic zone and to the African coast adds to the great productivity observed in the area. Dead cold-water scleractinean coral reefs, carbonate crusts and exposed carbonate chimneys characterise the Renard and Vernadsky Ridges. Carbonate crusts, rock blocks and clasts are often found in the craters of the shallow mud volcanoes where mild seepage activity has been recorded (Van Rensbergen *et al*. 2005).

Extensive authigenic carbonate provinces occur at intermediate depths (700-1200m) along the margins of Morocco and Spain. In the Spanish margin the Mediterranean outflow water interacts with the rough topography of diapiric ridges and exposes numerous carbonate chimney fields (e.g. in the Ibérico, Formosa and Guadalquivir Ridges and Cadiz Channel). In the Moroccan margin the carbonate provinces are accompanied by the frequent

occurrence of mounds, thickets and debris of mostly dead cold water scleractinean corals coral (e.g. around and at the flanks of the Meknès, Student and TTR mud volcanoes). This zone further includes other mud volcanoes from the western Moroccan field (e.g. Yuma, Ginsburg, Jesuz Baraza, Darwin, Chechaouan). The widespread presence of authigenic carbonates and also extensive *Neptunea* and *Bathymodiolus* graveyards (usually within the crater of the mud volcanoes) suggest that this was a very active seepage area in the past.

The deep-water field (1300-4000m), mostly within the Portuguese margin includes several mud volcanoes (e.g. Captain Arutyunov, Carlos Ribeiro, Bonjardim. Porto) that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz (Kenyon *et al.* 2000, 2001, 2002, 2003, 2006; Akhmetzhanov *et al*. 2007, 2008).

Sampling. Samples were collected between 2002 and 2006 during TTR12, TTR 14, TTR15 and TTR16 cruises onboard R/V Prof. Logachev and M.S. Merian 01/03 cruise onboard R/V Maria S. Merian (IFM-GEOMAR) (Table 2.4.1). The material was collected using TV-assisted grabs or USNEL box-corers. Occasionally faunal specimens were also recovered from Kasten-corer, multiple corer or lander samples that were carried out for different purposes. Whenever possible the specimens were sorted onboard and preserved in 70 or 96% ethanol (the latter preserved for molecular analysis).

Density and biodiversity. The total dataset of taxa was used to represent the regional biodiversity of the macrofauna, shown herein using accumulation, loss and turnover curves plotted against increasing depth (Colwell *et al*. 2004; Hurlbert 1971; Ugland *et al*. 2003).

Density (ind.m^{-2}) was calculated only for box-corer (BC) replicates and therefore restricted to a small number of samples from seven mud volcanoes: Mercator (3 BC collected during TTR15 and 2 BC taken during MSM01-03), Kidd (3 BC, TTR14), Meknès (2 BC, MSM01-03), Captain Arutyunov (2 BC, MSM01-03), Carlos Ribeiro (1 BC, MSM01-03), Bonjardim (1 BC, MSM01-03) and Porto (1 BC, MSM01-03). Rarefaction curves (Gotelli & Colwell 2001) were

plotted based in different quantitative samples taken from Mercator and Captain Arutyunov mud volcanoes.

2.4.3.Results

Species distribution and turnover. The sampling of the Gulf of Cadiz over the past few years provided a number of samples from which 62 yielded bivalves. The material examined was collected from 16 mud volcanoes and seven other locations in adjacent habitats (carbonate chimneys, crusts and mostly dead scleractinean thickets) and contained 759 specimens representing 49 species of 35 genera and 21 families (Table 2.4.1; Figures 2.4.1-2.4.3). Thyasiridae with eight species and Cuspidaridae with six species, were the best represented families. Most species were recorded in only one (46.5%) or a few samples (31.5% occurred in 2-5 samples) and thus the information on their distribution is fragmentary. The small-sized and common deep-sea bivalves *Abra longicallus*, *Microgloma pusilla* and *Limopsis minuta* were the most frequent species (in 16, 14 and 10 samples, respectively) followed by the seep-obligate *Petrasma* sp. nov.; *Acharax* sp. nov. and *Thyasira vulcolutre* (in 10, 9 and 9 samples, respectively) (Table 2.4.1).

Besides the chemosymbiotic species, all Thyasiridae species, some Cuspidaridae and to a lesser extent also the Nuculidae, Nuculanidae and Mytilidae occurred frequently associated to the mud breccia sediments in the crater of mud volcanoes (Table 2.4.1; Figure 2.4.4). On the other hand, all Pteriomorpha families (except for the Mytilidae) occurred exclusively in the adjacent habitats with carbonate chimneys, crusts and dead scleractinean thickets or at the flanks of mud volcanoes where such hard substrates were also found (Table 2.4.1; Figure 2.4.4).

Table 2.4.1. Station data for the Gulf of Cadiz samples yielding bivalves. Observations from TTR12 to TTR16 and MSM01-03 cruise reports (Kenyon *et al*. 2003, 2006; Pfannkuche 2006; Akhmetzhanov *et al*. 2007, 2008). D: Dredge; G: gravity core; Gr: TV-assisted grab; UB: USNEL box core; MC: Multi core; K: Kasten core; BL: BIGO lander; FL: FLUFO lander.

Structure	Cruise	Station	Gear	Date dd.mm.yy	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
Mud volcanoes									
Al Idrisi	TTR12	AT412	D	17.07.2002	35º14.193′	06º36.609′	230	*B. asperula*	Crater; sandstones
Mercator	TTR15	AT569	Gr	25.07.2005	35º17.917′	06º38.717′	358	*Petrasma* sp.nov.; *E. aegeensis; Lucinoma* sp. nov.	Crater; mud breccia, near seep
		AT575	UB	26.07.2005	35º17.903′	06º38.715′	355	*E. aegeensis, M. ferruginosa;* Thyasiridae und.; *A. longicallus; V. atlantica*	Crater; mud breccia; near seep
		AT576	UB	26.07.2005	35º17.657′	06º39.129′	428	*A. croulinensis;* Thyasiridae und.; *K. abyssicola*	Flank; mud breccia
		AT577	UB	26.07.2005	35º17.305′	06º39.672′	485	*E. aegeensis; M. pusilla; A. longicallus; K. abyssicola;* Cuspidaridae und.	Off MV; hemipelagic sediments
	MSM01-03	237.2	MC	06.05.2006	35º17.914′	06º38.687′	353	*M. pusilla*	Crater; mud breccia, near seep
		241	UB	06.05.2006	35º17.918′	06º38.717′	353	*E. aegeensis; N. sulcata; M. pusilla; Nuculoma* sp.; *T. granulosa, T. obsoleta; A. croulinensis; A. longicallus; C. abbreviata;* Bivalvia und.	Crater; mud breccia, near seep; *Caryophyllia* facies
		242	UB	06.05.2006	35º17.870′	06º38.810′	350	*E. aegeensis; T. granulosa; A. croulinensis; L. minutus; A. longicallus*	Crater; mud breccia *Hyalonecia* facies
		287	MC	11.05.2006	35º17.890′	06º39.059′	379	*A. croulinensis;* Thyasiridae und	Crater rim; mud breccia
Fiúza	TTR14	AT566	Gr	09.08.2004	35º15.510′	06º41.702′	414	*Petrasma* sp.nov.; *M. pusilla*	Crater; mud breccia, and marl
Kidd	TTR14	AT528	Gr	03.08.2004	35º25.304′	06º43.972′	489	*Petrasma* sp.nov.; *A. longicallus*	Crater;mud breccia
		AT559	UB	08.08.2004	35º24.777′	06º43.782′	552	*E. bushae; M. pusilla; M. tumidula;* Thyasiridae und.; *A. longicallus*	Off MV; hemipelagic sediments
		AT560	UB	08.08.2004	35º25.306′	06º43.976′	498	*Petrasma* sp.nov.; *M. pusilla; L. messanensis; A. longicallus ; K. abyssicola*	Crater; mud breccia
		AT561	UB	08.08.2004	35º25.602′	06º44.099′	526	*M. pusilla; K. abyssicola*	Flank;hemipelagic sediments
TTR	TTR12	AT416	Gr	17.07.2002	35º21.870′	06º52.000′	695	*L. messanensis, B. phillipiana; B. asperula; L. minuta; D. vitreus; C.*	Flank; coral framework

Structure	Cruise	Station	Gear	Date dd.mm.yy	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
								hoskynsi; L. excavata	
Meknès	TTR15	AT581	Gr	28.07.2005	34º59.178′	07º04.353′	700	Bivalvia und	Crater; mud breccia
		AT586	Gr	28.07.2005	34º59.146′	07º04.380′	701	*Petrasma* sp.nov.	Crater; mud breccia
	MSM01-03	319	UB	14.05.2006	34º59.100′	07º04.439′	695	*A. croulinensis; A. longicallus*	Crater; mud breccia
		321	UB	14.05.2006	34º58.796′	07º04.394′	732	*T. granulosa*	Carbonate mound; shell ash and coral debris
		335	UB	15.05.2006	34º59.035′	07º04.552′	703	*D.* cf *maneni; K. abyssicola; C. nflata;* Cuspidaridae und.	Flank; coral debris, mud breccia
Student	TTR10	AT239	Gr	22.07.2000	35º30.853'	07º08.816'	955	*L. minuta*	
Yuma	TTR14	AT524	Gr	02.08.2004	35º24.973′	07º05.461′	960	*Petrasma* sp.nov.	Crater;,mud breccia
	TTR16	AT604	Gr	29.05.2006	35º25.820′	07º06.330′	1030	*Petrasma* sp.nov.; *Acharax* sp. nov.; *L. minutes; Cuspidaria sp*A; Bivalvia und	Flank;mud breccia
		AT605	Gr	29.05.2006	35º25.046′	07º05.450′	975	*Petrasma* sp.nov.; *A. longicallus*	Cratter; mud breccia
Ginsburg	TTR16	AT607	Gr	29.05.2006	35º22.677′	07º04.979′	983	*Petrasma* sp.nov.; *Acharax* sp. nov.; *E. bushae*	Shell ash;, carbonate slabs, crusts and coral debris
Jesuz Baraza	TTR12	AT391	Gr	09.07.2002	35º35.439′	07º12.264′	1105	*Acharax* sp. nov.; *M. pusilla; M. tumidula; Poromya* sp.	
Darwin	TTR16	AT608	Gr	30.05.2006	35º23.531′	07º11.475′	1115	*Petrasma* sp.nov.; *E. bushae; D. balgimi; B. mauritanicus; L. minutus; A. longicallus; C. nflata*	Crater; shell ash; slabs and crusts.
…Chechaouen	TTR16	AT610	Gr	30.05.2006	35º28.468′	07º15.477′	1177	*T. granulosa; K. abyssicola; C. inflata;* Cuspidaridae und..; Bivalvia und.	Crater; mud breccia (clasts, carbonate crusts)
Cap. Arutyunov	TTR12	AT393	Gr	09.07.2002	35º39.740'	07º19.942'	1327	*M. pusilla*	
	TTR12	AT399	Gr	13.07.2002	35º39.805′	07º19.997′	1339	*M. pusilla; M. tumidula; Propeamussium* sp.; Cuspidaridae und.	Crater rim; mud breccia
	TTR14	AT548	K	06.08.2004	35º39.708'	07º19.034'	1345	*T. granulosa*	Crater; mud breccia with gas hydrates
	MSM01-03	180	UB	27.04.2006	35º39.740'	07º19.960'	1323	*Acharax* sp. nov.; *Dacrydium* sp.; *A. croulinensis; I* cf. *Perplexum; Cuspidaria sp*C; Bivalvia und	
		190.1	MC	28.04.2006	35º39.665'	07º19.970'	1322	*S. sentosus*	
		190.2	MC	28.04.2006	35º39.668'	07º19.970'	1320	*T. vulcolutre; T. obsoleta*	
		195	Gr	28.04.2006	35º39.274′	07º20.013′	1390	*E. aegeensis; B. phillipiana; B. asperula, D. balgimi; Cuspidaria sp*A	Flank; coral thicket
		212	BL	30.04.2006	35º39.681'	07º19.981'	1317	*N. tumidula; M. pusilla; C. inflata;* Bivalvia und	
		217	UB	30.04.2006	35º39.642'	07º20.049'	1321	*T. vulcolutre; Isorropodon* nov. sp.	
		225	BL	04.05.2006	35º39.700′	07º20.010′	1320	*M. pusilla; T. vulcolutre; A. longicallus; Isorropodon* nov. sp.	Crater center; mud breccia

Structure	Cruise	Station	Gear	Date dd.mm.yy	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
		274	BL	10.05.2006	35º39.706′	07º20.001′	1321	*Acharax* sp. nov.; *M. pusilla; T. vulcolutre; A. croulinensis ; A. longicallus*	Crater center; mud breccia
		344	FL	16.05.2006	35º39.697′	07º20.038′	1320	*T. vulcolutre;* Thyasiridae und	Crater; mud breccia
Carlos Ribeiro	TTR10	AT243	G	26.07.2000	35º47.217'	08º25.313'	2200	*T. vulcolutre*	
	TTR16	AT615	Gr	31.05.2006	35º47.238′	08º25.272′	2200	*Acharax* sp. nov.; *T. vulcolutre; C. inflata*	
		AT617	K					*Acharax* sp. nov.	
	MSM01-03	157	UB	23.04.2006	35º47.270′	08º25.360′	2200	*M. johnsoni; B. asperula; T. vulcolutre*	Crater; mud breccia
		169	MC	25.04.2006	35º47.256'	08º25.361'	2199	*T. vulcolutre; T. obsoleta*	
		184	FL	27.04.2006	35º47.260'	08º25.346'	2200	*M. johnsoni*	
Bonjardim	MSM01-03	133	UB	19.04.2006	35º27.821'	09º00.128'	3049	*P. nitens, P. latior*	
Porto	TTR16	AT622	Gr	03.06.2006	35º33.773′	09º30.416′	3902	*M. pusilla; T. tortuosa*	Crater; mud breccia
	MSM01-03	145	UB	21.04.2006	35º33.699'	09º30.437'	3860	*Acharax* sp. nov.; *Microgloma* sp. *Y. inconspicua; T. obsoleta*	
		161	MC	24.04.2006	35º33.754'	09º30.499'	3864	Mytilidae und.	Crater; mud breccia
Carbonates and Corals									
W of Gibraltar	TTR14	AT550	D	07.08.2004	35º42.257′	06º30.000′	392	*S. gussoni*	Carbonate chimneys
		AT551	D	07.08.2004	35º42.769′	06º30.305′	393	*B. asperula; L. minuta; C. hoskynsi; S. gussoni*	Carbonate chimneys
		AT552	Gr	07.08.2004	35º42.816′	06º30.234′	428	*M. tumidula; L. minuta; S. gussoni;* Cuspidaridae und.	Carbonate chimneys in brawn marl
Vernadsky R.	TTR15	AT574	D	26.07.2005	35º02.982′	06º46.661′	508	*E. bushae; L. minuta*	Carbonate crusts and chimneys
Ibérico	TTR11	AT335	D	25.08.2001	36º07.405'	07º41.198'	905	*B. asperula; S. gussoni; H. squamala*	Carbonate chimneys
Guadalquivir R.	TTR11	AT339	D	26.08.2001	36º07.591'	07º46.587'	1021	*H. artica*	Carbonate chimneys
Formosa Ridge	TTR12	AT388	Gr	08.07.2002	36º10.263′	07º43.819′	1079	*B. asperula; L. minuta*	Carbonate chimneys
		AT389	D	08.07.2002	36º10.123′	07º44.121′	1068	*B. asperula, L. minuta*	Carbonate chimneys
Cadiz Channel	TTR15	AT599	D	03.08.2005	36º06.538'	07º53.942'	1275	*Cuspidaria* spB	
Pen Duick Escarpment	TTR12	AT406	Gr	15.07.2002	35º18.148′	06º47.666′	550	*M. tumidula; D. vitreus; A. longicallus*	Coral framework
		AT407	Gr	15.07.1002	35º17.695′	06º47.082′	560	*L. minuta*	Coral framework
	TTR16	AT600	Gr	28.05.2006	35º18.779′	06º48.453′	610	*E. bushae, B. phillipiana; L. minuta; D. vitreus; S. gussoni; A. longicallus; K. abyssicola*	Mud breccia covered by coral debris and carbonate crusts
		AT602	Gr	28.05.2006	35º17.693′	06º47.089′	556	*Acharax* sp. nov.; *E. bushae; A. longicallus*	Hemipelagic sediments, shell ash cemented by carbonate

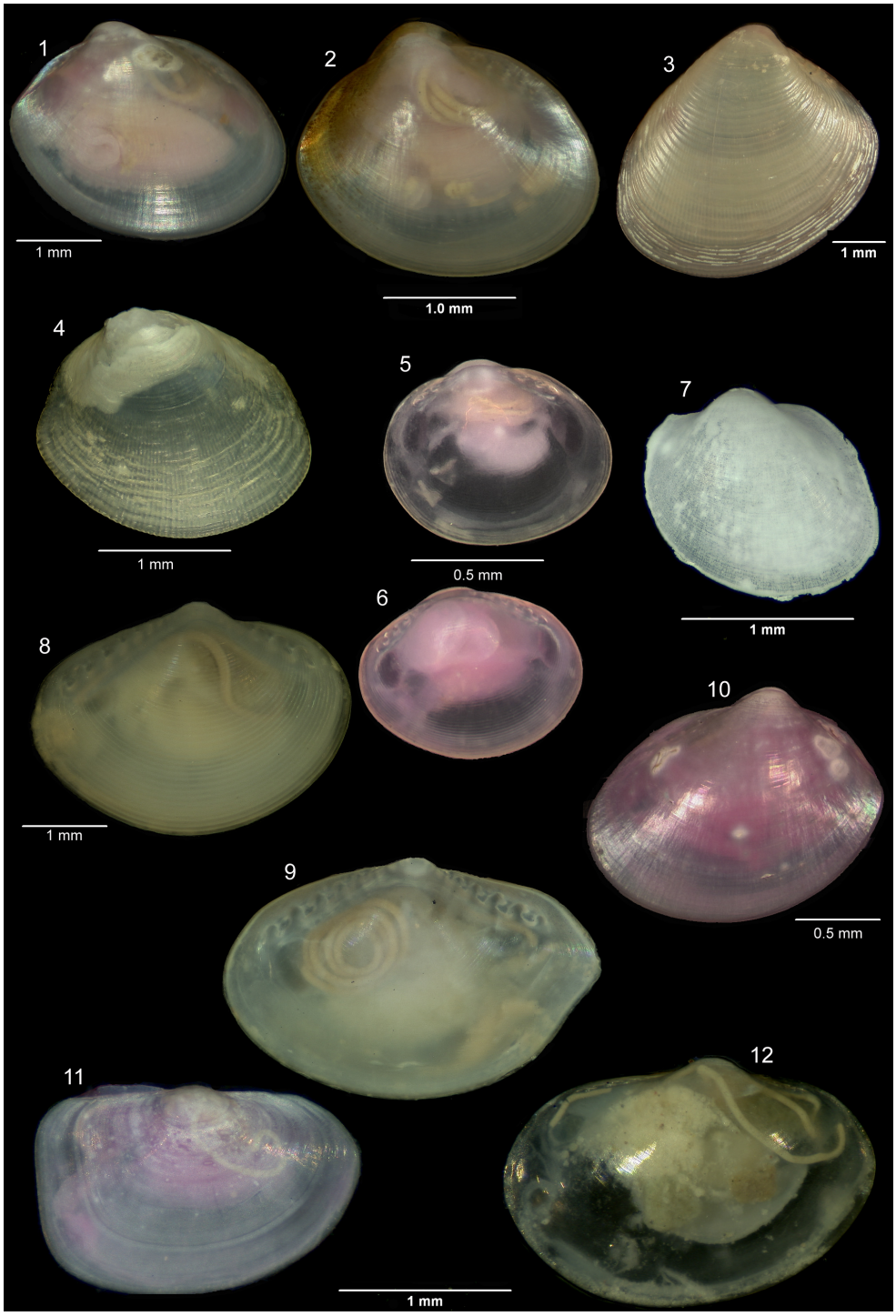

Figure 2.4.1. 1: *Ennucula aegeensis*, 2: *Ennucula bushae*, 3: *Nucula tumidula*, 4: *Nucula sulcata*, 5: *Microgloma tumidula*, 6: *Microgloma pusilla*, 7: *Pristigloma nitens*, 8: *Pseudoneiloneilla latior*, 9: *Ledella messanensis*, 10: *Nucoloma* sp., 11: *Malletia johnsoni*, 12: *Yoldiella inconspicua* sensus Allen.

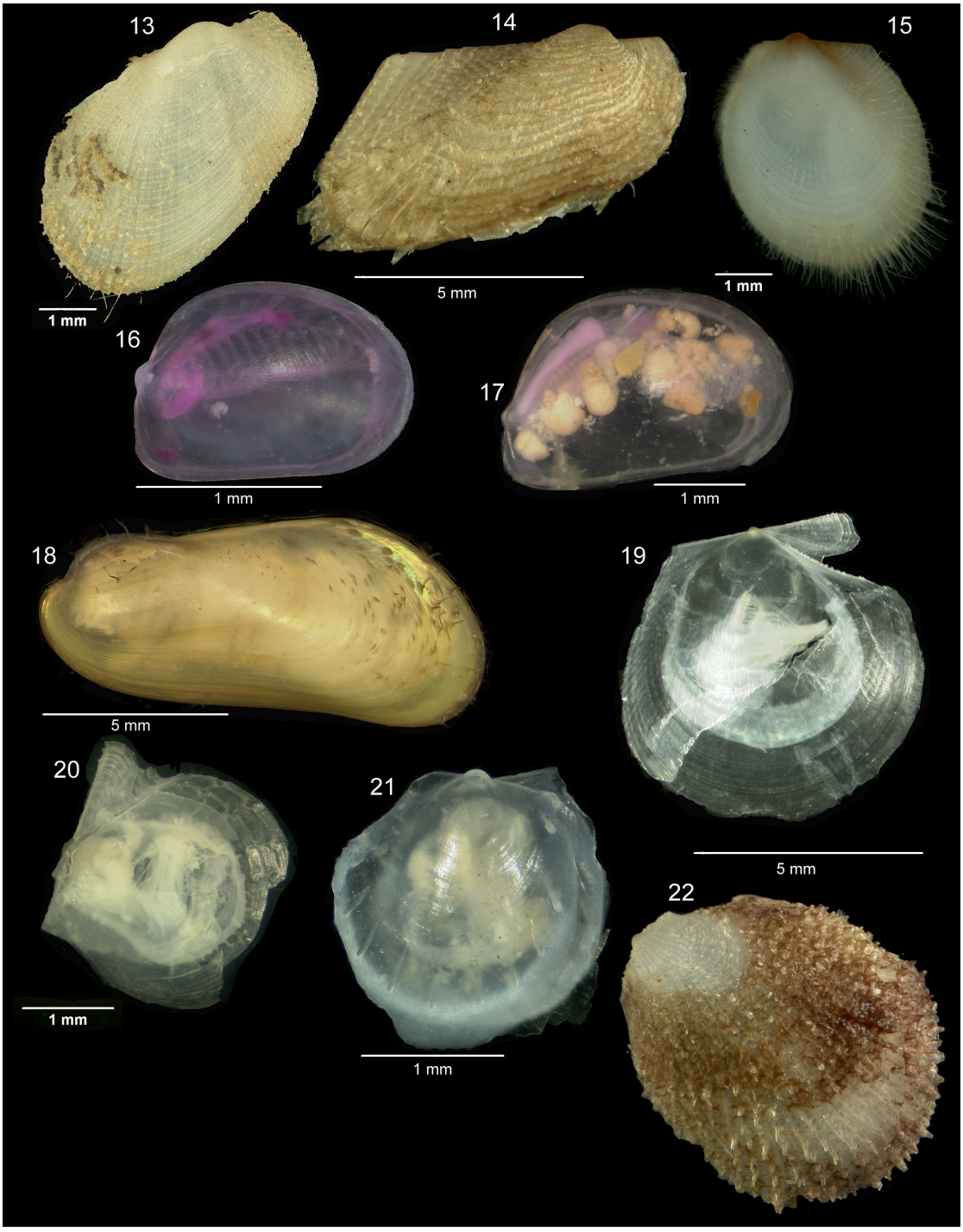

Figure 2.4.2. 13: *Bathyarca phillipiana*, 14: *Bentharca asperula*, 15: *Limopsis minuta*, 16: *Dacrydium balgimi*, 17: *Dacrydium* cf. *maneni*, 18: *Bathymodiolus mauritanicus*, 19: *Delectopecten vitreus*, 20: *Cyclopecten hoskynsi*, 21: *Propeamussium* sp., 22: *Spondylus gussoni*.

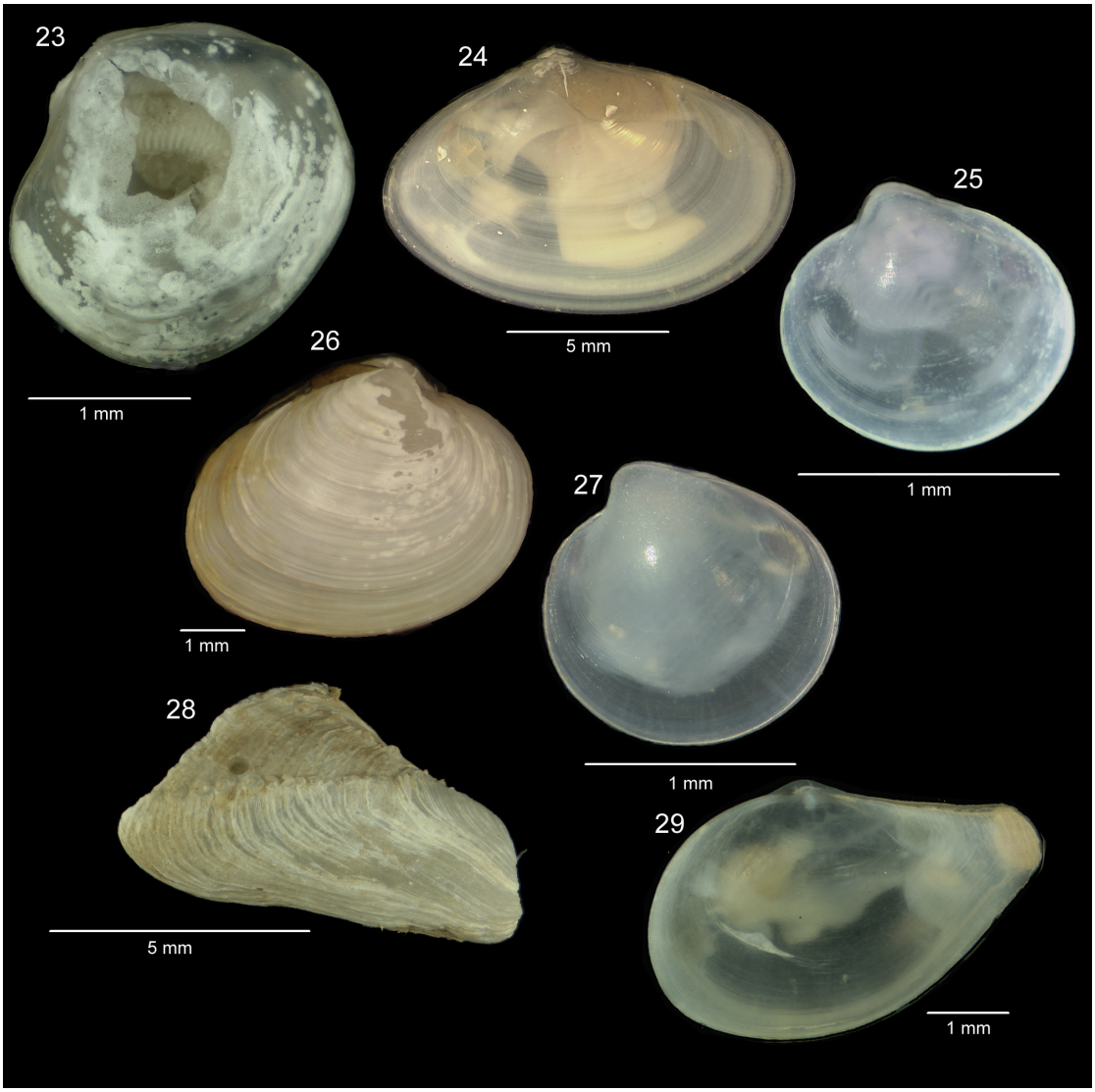

Figure 2.4.3. 23: *Spinaxinus sentosus*, 24: *Abra longicallus*, 25: *Kelliella abyssicola*, 26: *Isorropodon* sp. nov, 27: *Vesicomya atlantica*, 28: *Hiatella artica*, 29: *Cuspidaria inflata*.

A similar number of species (25 to 27) occurred in each of the three areas studied.

The El Arraiche area (Moroccan margin; 250-650m) is characterized by the species *Ennucula aegeensis*, *Ennucula bushae*, *Microgloma pusilla*, *Microgloma tumidula*, *Axinulus croulinensis*, *Abra longicallus* and *Kelliella abyssicola*. The area includes one of the best sampled mud volcanos, the Mercator with a total of 15 bivalve species from which five occur as singletons (*Nucula sulcata*, *Nuculoma* sp., *Mendicula ferruginosa*, *Vesicomya atlantica* and *Cuspidaria abbreviata*). *Lucinoma* sp. nov and *Petrasma* sp. nov are the obligate seep species present in the El Arraiche mud volcano field.

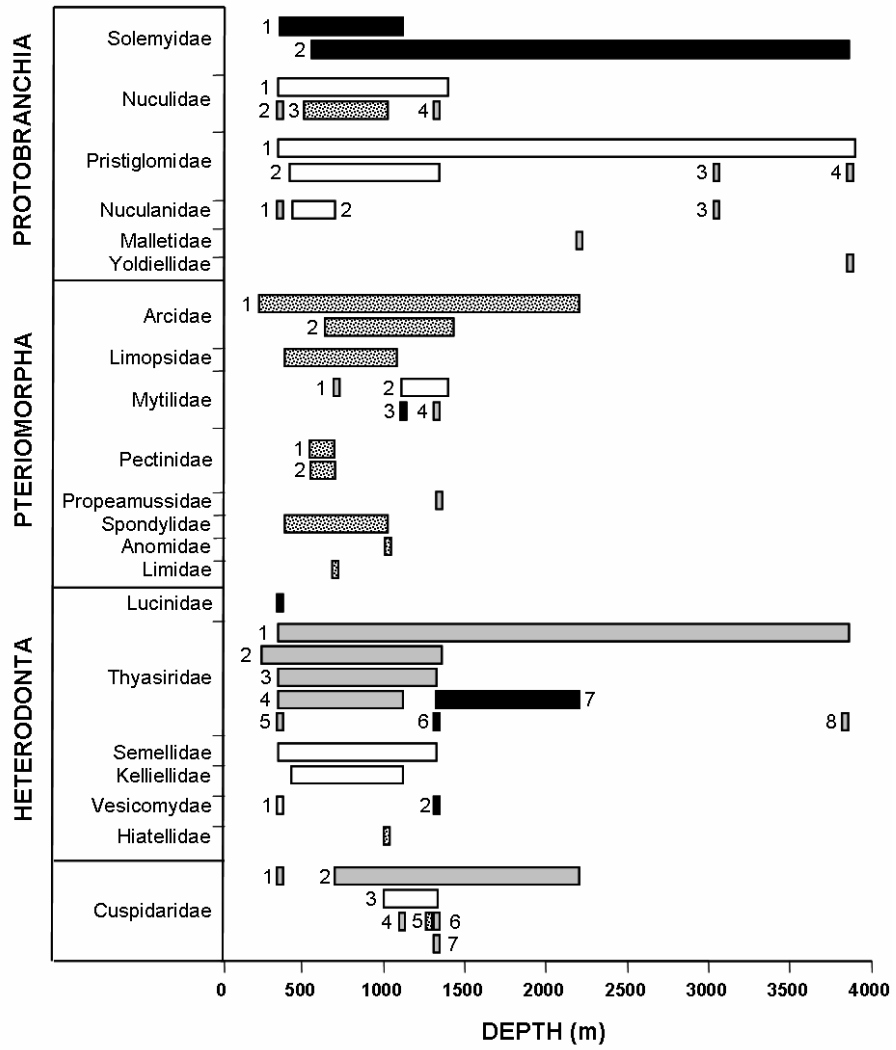

Figure 2.4.4. Bathymetric ranges of the bivalve families collected in the Gulf of Cadiz. **Solemyidae**: 1. *Petrasma* sp. nov., 2. *Acharax* sp. nov.; **Nuculidae**: 1. *Ennucula aegeensis*; 2. *N. sulcata*, 3. *E. bushae*, 4. *N. tumidula*; **Pristoglomidae**: 1. *M. pusilla*, 2. *M. tumidula*, 3. *P. nitens*, 4. *Microgloma* sp.. **Nuculanidae**: 1. *Nuculoma* sp., 2. *L. messanensis*; 3. *P. latior;* **Malletidae**: *M. johnsoni*; **Yoldiellidae**: *Y. inconspicua* sensus Allen; **Arcidae**: 1. *B. asperula*, 2. *B. phyllipiana*; **Limopsidae**: *L. minuta*; **Mytilidae**: 1. *D.* cf. *maneni,* 2. *D. balgimi,* 3. *B. mauritanicus,* 4. *Dacrydium* sp.; **Pectinidae**: 1. *C. hoskynsi,* 2. *D. vitreus;* **Propeamussidae**: *Propeamussium* sp.; **Spondylidae**: *S. gussoni*; **Anomidae**: *H. squamala*; **Limidae**: *L. excavata*; **Lucinidae**: *Lucinoma* sp. nov.; **Thyasiridae**: 1. *T. obsoleta,* 2. *T. granulosa,* 3. *A. croulinensis,* 4. *L. minutus,* 5. *M. ferruginosa,* 6. *S. sentosus,* 7. *T. vulcolutre,* 8. *T. tortuosa;* **Semellidae**: *A. longicallus;* **Kelliellidae**: *K. abyssicola*; **Vesicomyidae**: 1. *V. atlantica*; 2. *I. perplexum* and *Isorropodon* sp. nov.; **Hiatellidae**: *H. artica;* **Cuspidaridae**: 1. *C. abbreviata,* 2. *C. inflata,* 3. Cuspidaria spA, 4. Cuspidaria spB, 5. Cuspidaria spC, 6. Cuspidaria spD; 7, Cuspidaria spE. Black bars: Chemosymbiotic species; White bars: Species occurring at all settings; Grey bars: Species found in sediments of the mud volcanoes' craters; Stippled bars: Species found in assocation with carbonate concretions and cold water corals.

The second area includes the extensive carbonate provinces of the Spanish and Moroccan margins at a depth range of 700 to 1200m. It is characterized mainly by Pteriomorpha species, either found exclusively in this area (*Dacrydium* cf. *maneni*, *Heteranomia squamala* and *Lima excavata*) or shared with the shallower El Arraiche field (*Bentharca asperula*, *Bathyarca phillipiana*, *Limopsis minuta*, *Cyclopecten hoskynsi*, *Delectopecten vitreus* and *Spondylus gussoni*). *Petrasma* sp. nov., *Acharax* sp. nov and *Bathymodiolus mauritanicus* are the obligate seep species found in the mud volcanoes of the Western Moroccan field.

The deeper area encompasses four very active mud volcanoes located at depths between 1300 and 4000m, including Captain Arutyunov where the highest number of bivalve species (21) and specimens (276) were collected. A much lower number of species and specimens were obtained from the samples collected at the three deeper mud volcanoes (only 30 specimens representing 12 out of the total 27 species in the area). As in the shallower mud volcanoes of the El Arraiche field, the bivalve assemblage includes the families Nuculidae, Pristoglomidae and Thyasiridae and Cuspidaridae although with different representative species. Many species occur as singletons in this deep area (e.g. *Nucula tumidula*, *Microgloma* sp., *Pristigloma nitens*, *Pseudoneiloneilla latior*, *Malletia johnsoni*, *Yoldiella inconspicua*, *Thyasira tortuosa*, *Dacrydium* sp. and *Cuspidaria* sp.) but the most distinctive feature of the bivalve assemblage is the high number of obligate seep species. Besides the more frequent *Acharax* sp. nov. and *Thyasira vulcolutre* three other species occur in Captain Arutyunov (*Spinaxinus sentosus*, *Isorropodon* sp. nov. and *Isorropodon perplexum*) and another species recently collected from Bonjardim awaits further study (*Calyptogena* cf. *regab*). The known bathymetric ranges and trophic status of the species found in this study are shown in Table 2.4.2.

The discontinuities in the species accumulation curve (Figure 2.4.5) reflect the fragmentary distribution of many species and major gaps in the data set at greater depths where few mud volcanoes were sampled. Nevertheless, in many cases different families in each Sub-Class, or species in each Family, showed segregated bathymetric distributions (Figure 2.4.4) resulting in

different areas of enhanced turnover that can be identified (Figure 2.4.5). The shallow El Arraiche area is marked by the initial high rate of species addition owing to the elevated species richness of Mercator (15 species), followed by a steady increase in species and combined with a low loss rate. This trend is interrupted at 650-700m depth by a sudden increase in the rate of species loss. The zone extending from 700 to 1200m is characterized by the irregularity of the accumulation curve and by the high rate of loss, exceeding almost twofold the rate of addition (nine species added against sixteen lost). The major turnover in the bivalve assemblage occurs at 1300m at Captain Arutyunov where up to eight species from the shallower zones have their deepest boundary. Faunal turnover is further enhanced by the seven species restricted to this mud volcano and an additional one that extends to greater depths. Except for the Captain Arutyunov the few isolated mud volcanoes at greater depths were poorly sampled, which could account for the very low rate of species addition from ~1300 to 4000m (Figure 2.2.5).

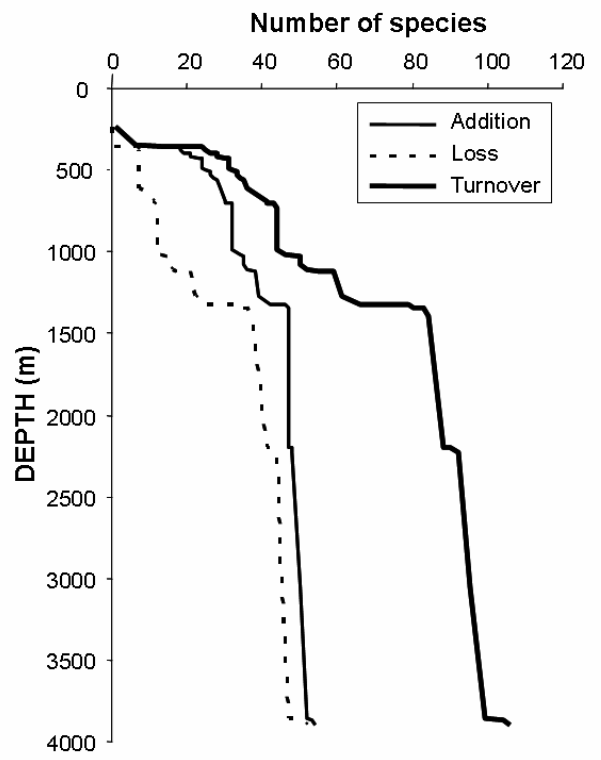

Figure 2.4.5. Regional species accumulation, loss and turnover curves along the depth gradient in the Gulf of Cadiz.

Abundance and structure of the assemblages. The analysis of a few quantitative samples (USNEL box cores) evidenced the high heterogeneity of the studied assemblages at different spatial scales. Samples taken within the crater of a single mud volcano, at different zones of the mud volcano or at different mud volcanoes showed a wide variation in abundance, species richness and both taxonomic and trophic structure of the assemblages (Figures 2.4.6, 2.4.7 and 2.4.8).

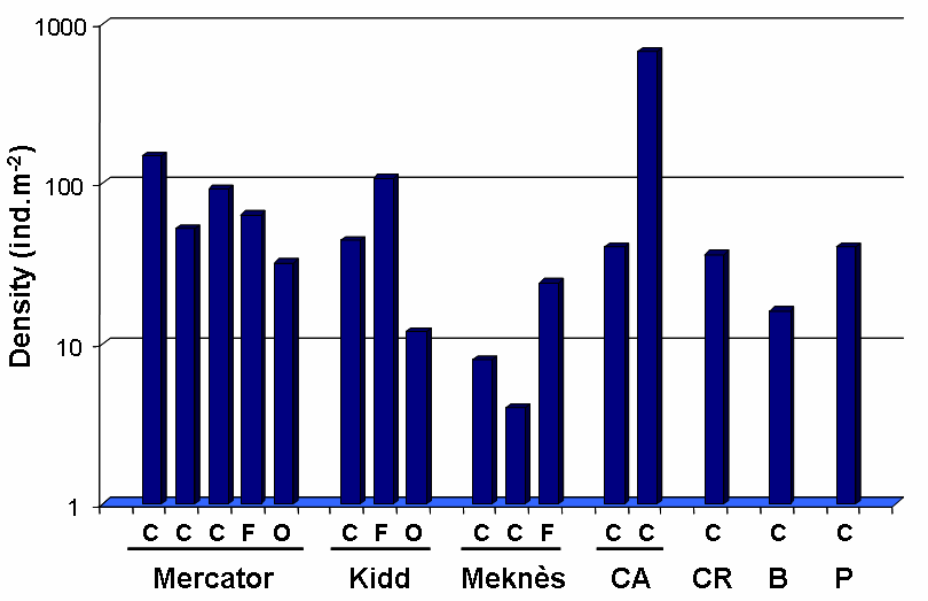

Figure 2.4.6. Macrofaunal taxa density in individual samples (USNEL boxcore; Area: 0.25m^2) from seven different mud volcanoes in the Gulf of Cadiz. CA: Captain Arutyunov; CR: Carlos Ribeiro; Por: Porto.

Bivalve densities estimated from single box core samples (Area=0.25m^2) ranged usually from 10 to 100 ind.m^{-2}, with extreme values of 4 and 664 ind.m^{-2} in Meknès and Captain Arutyunov, respectively (Figure 2.4.6). The number of bivalve species per sample varied between two and five (Figure 2.4.7) with the lowest values in Meknès (only one species), and the highest in Captain Arutyunov and Mercator (7 and 10 species, respectively). In fact, Meknès was the mud volcano with the poorest bivalve assemblage with low species richness (1-4 species per sample; 8 species in total) and abundance (4-24 ind.m^{-2}) while Mercator (up to 10 species per sample, 15 species in total and densities up to 148 ind.m^{-2}) and Captain Arutyunov (up to 7 species per sample, 21 species in total and densities up to 664 ind.m^{-2}) showed the richest

bivalve assemblages. The trophic structure of the assemblages also varied from one mud volcano to another showing a trend to a greater contribution of chemosymbiotic species in the deeper mud volcanoes both in terms of number of species and abundance (Figures 2.4.7 and 2.4.8). In Mercator, suspension feeders and species with alternative feeding strategies (chemotrophic vs suspension feeding) dominated the assemblage while at Kidd and at the deepest mud volcanoes (Carlos Ribeiro, Bonjardim and Porto) there was an enhanced contribution of deposit feeders. Predators, essentially represented by the family Cuspidaridae, showed no clear pattern related either to depth or spatial zonation (Figure 2.4.8).

Samples taken in the crater, flank and off mound in Mercator and Kidd showed lower species richness and abundance off mound (Figures 2.4.6 and 2.4.7) but this trend must be interpreted with caution given the lack of replicate sampling. As it might be expected obligate chemotrophic species were only present in the crater and absent from the assemblages off mound but some species with alternative feeding strategies (chemotrophic vs suspension feeding) occurred both in the crater and flanks of the mud volcanoes although with lower percentual contributions in the latter (Figures 2.4.7 and 2.4.8).

The two samples taken from Captain Arutyunov are an example of the high variability that may occur within the crater of a single mud volcano. One sample characterized by the presence of a large amount of gas hydrates, was completely dominated by high numbers of only two chemotrophic species, *Isorropodon* sp. nov. and *Thyasira vulcolutre*. In the other sample the density of bivalves was one order of magnitude lower and the occurrence of gas hydrates was not evident. Chemotrophic species (*Isorropodon* sp. nov. and *Petrasma* sp. nov.) accounted for 50% of the total abundance but five other species contributed evenly to the remaining 50% (Figures 2.4.6, 2.4.7 and 2.4.8)

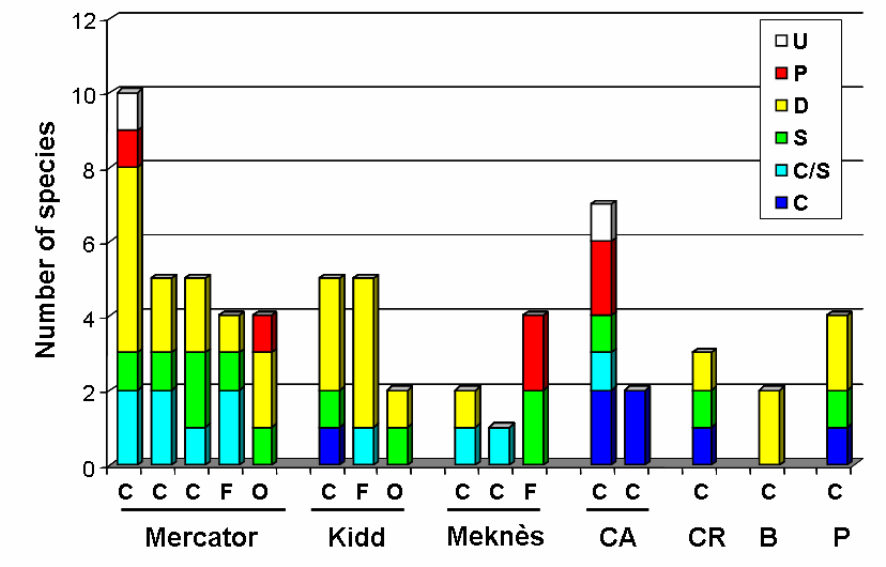

Figure 2.4.7. Macrofaunal taxa richness and different trophic groups in individual samples (USNEL boxcore; Area: 0.25m^2) from seven different mud volcanoes in the Gulf of Cadiz. Trophic groups: C. Chemosynthetic; C/S. Chemosynthetic and/or suspension-feeder; S. suspension feeder; D. Deposit feeder; P. Predator; U. Unkown. Mud volcanoes: CA. Captain Arutyunov; CR. Carlos Ribeiro; Por. Porto. Sites: C. Crater; F. Flank; O. Off-mound.

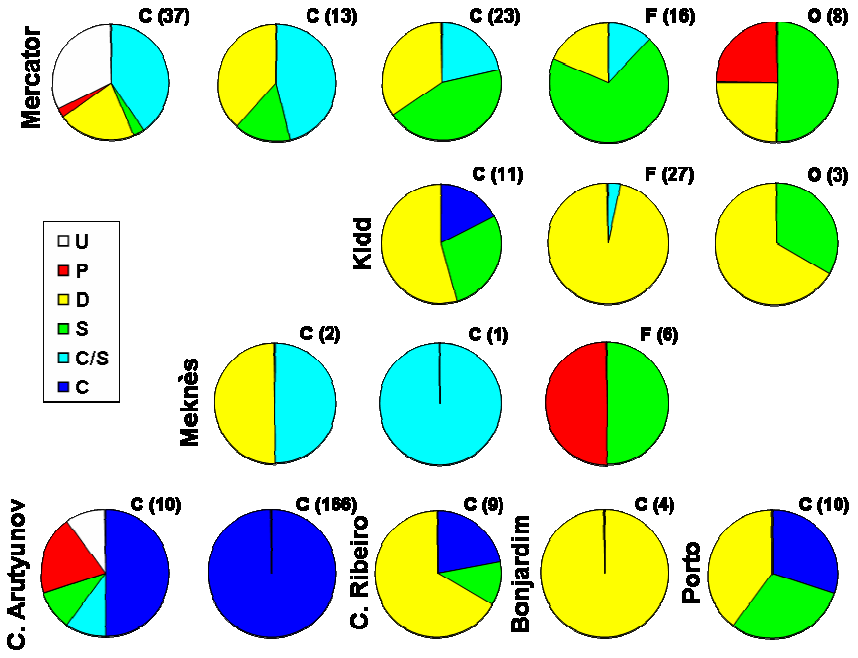

Figure 2.4.8. Trophic structure of the bivalve assemblages collected in individual samples (USNEL boxcore; Area: 0.25m^2) from seven different mud volcanoes in the Gulf of Cadiz; numbers in brackets indicate the total number of specimens in each sample. Trophic groups: C. Chemosynthetic; C/S. Chemosynthetic and/or suspension-feeder; S. suspension feeder; D. Deposit feeder; P. Predator; U. Unkown. Sites: C. Crater; F. Flank; O. Off-mound.

The diversity of the bivalve assemblage in Mercator and Captain Arutyunov was compared using Hulberts' expected number of species plotted against the sampled number of specimens (Figure 2.4.9). Several quantitative samples (USNEL box cores but also smaller sample from lander chambers and multicore replicates) were available for both mud volcanoes and represented a similar sampled area (approx. 0.8m^2) for each mud volcano. Interestingly the same sampled area yielded a similar number of species (15) in both mud volcanoes but the diversity was considerably higher in Mercator owing to the greater evenness of the assemblage. In Captain Arutyunov the high densities of chemotrophic species that dominate the assemblage explain its lower diversity.

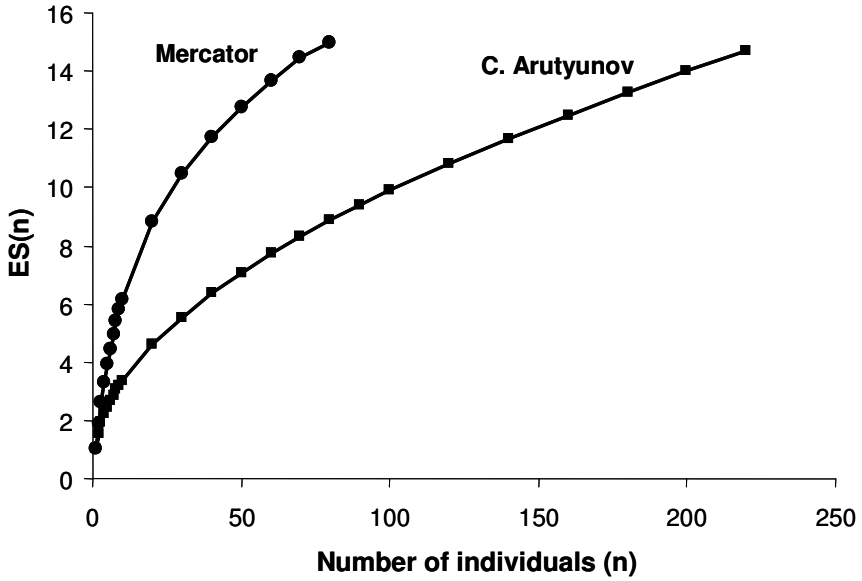

Figure 2.4.9. Species rarefaction curves for Mercator and Captain Arutyunov mud volcanoes.

Table 2.4.2. Bivalvia presence in the the Gulf of Cadiz. N. Total number of specimens collected; TG.Trophic groups; C. Chemosynthetic; C/S. Chemosynthetic and/or suspension-feeder; S. suspension feeder; D. Deposit feeder; P. Predator; U. Unkown. a) this study; Génio *et al.* 2008, Rodrigues *et al* 2008; b) Cosel & Salas 2001; Allen & Sanders 2001; Olabarria 2005; Salas 2005.

Family	**Species**	**N**	**TG**	**Depth (m) a)**	**Depth (m) b)**
Undetermined	Undetermined	28			
Solemyidae	*Petrasma* sp. nov.	78	C	328-1115	-
	Acharax sp. nov.	15	C	556-3860	-
Nuculidae	*Ennucula aegeensis*	8	D	350-1390	155-1200
	Ennucula bushae	14	D	508-1115	530-4630
	Nucula tumidula	1	D	1322	1255-1527
	Nucula sulcata	1	D	353	56-398
Pristoglomidae	*Microgloma tumidula*	25	D	426-1339	722-1523
	Microgloma pusilla	30	D	353-3902	740-1523
	Microgloma sp	3	D	3860	-
	Pristigloma nitens	3	D	3049	1021-4853
Nuculanidae	*Pseudoneiloneilla latior*	1	D	3049	
	Ledella messanensis	1	D	438-695	425-1805
	Nuculoma sp.	2	D	353	-
Malletiidae	*Malletia johnsoni*	7	D	2200-2223	1100-3834
Yoldiidae	*Yoldiella inconspicua* sensus Allen	1	D	3860	700-4000
Arcidae	*Bathyarca phillipiana*	1	S	610-1390	135-546
	Bentharca asperula	65	S	230-2200	2028-2100
Limopsidae	*Limopsis minuta*	36	S	393-1079	309-1700
Mytilidae	*Dacrydium balgimi*	23	S	1115-1390	385-1510
	Dacrydium cf *maneni*	1	S	703	
	Dacrydium sp.	1	S	1323	-
	Undetermined	1			-
	Bathymodiolus mauritanicus	14	C	1115	
Pectinidae	*Delectopecten vitreus*	3	S	550-695	395-4100
	Cyclopecten hoskynsi	2	S	393-695	250-546
Propeamussidae	*Propeamussium* sp.	2	S/P	1339	-
Spondylidae	*Spondylus gussoni*	10	S	392-1021	365-580
Anomidae	*Heteranomia squamula*	1	S	1021	
Limidae	*Lima excavata*	1	S	393-695	
Lucinidae	*Lucinoma* sp. nov.	19	C	358	-
Thyasiridae	*Thyasira vulcolutre*	50	C	1320-2200	1320
	Thyasira granulosa	9	C/S	350-1345	100-1800
	Thyasira tortuosa	1	U	3902	
	Thyasira obsoleta	6	S	353-3860	24-4100
	Axinulus croulinensis	24	C/S	350-1323	40-3800
	Mendicula ferruginosa	9	S	355	50-4825
	Leptaxinus minutus	5	S	350-1115	
	Spinaxinus sentosus	1	C	1320	
	Undetermined	9			
Semelidae	*Abra longicallus*	33	D	350-1321	290-1470
Kelliellidae	*Kelliella abyssicola*	24	S	428-1117	116-1523
Vesicomyidae	*Isorropodon* sp. nov.	143	C	1320-1322	-
	Isorropodon perplexum	1	C	1320	2420
	Vesicomya atlantica	1	S	355	1800-3225
Hiatellidae	*Hiatella artica«*	1	S	1021	16-523
Cuspidaridae	*Cuspidaria abbreviata*	1	P	353	181-555
	Cuspidaria inflata	9	P	703-2200	948-1870
	Cuspidaria A	3	P	1030-1330	-
	Cuspidaria B	1	P	1275	-
	Cuspidaria C	1	P	1323	-
	Undetermined	8			-
	Poromya sp.	1	P	1105	-

2.4.4.Discussion

The Southern Iberian Peninsula shows a highly diverse bivalve assemblage. Salas (1996) mentions 239 species from which 105 occur at the slope between the shelf break and depths ~200m From the 49 species reported herein only twenty were also reported by Salas (1996). Thse are considerably high species numbers compared to reports from the Porcupine Seabight, NE Atlantic, of 76 species from depths between 500 and 4866m (Olabarria 2005) and from the continental slope off New Jersey NW Atlantic, of 45 species from 1500-2500m (Grassle & Maciolek 1992). The number of Protobranch species reported by Allen & Sanders (1996) for the Western Eurpean basin (NE Atlantic) is less than 40 only a litlle more than the 15 protobranch species reported here for the Gulf of Cadiz alone.

Part of the explanation of the high biodiversity of the area is supported by the geologic history of the central Atlantic that started some 180-160 Ma ago with the formation of the Tethys Seaway. The colonization of the deep Atlantic, discussed by Allen & Sanders (1996) is hypothesized to have had four major points of entry from which the earliest and most important was Tethyan. According to these authors, the present highest diversity of the Western European basin reflects the fauna's ancient Tethyan origin supplemented later by othter additions of deep water species. The Gulf of Cadiz (the present day reminiscent of the ancient Tethyan point of entry) is therefore one of the older, if not the oldest deep-sea regions of the Western basin.

Besides the long-term diversity that involves the evolution over geological time spans also short-term diversity predominantly in physically controlled environments among which deep-sea reducing environments such as vents and seeps can be included. Cold seeps are among the most heterogeneous of all continental margin ecosystems. Sources of heterogeneity in cold seeps may arise from local variability in fluid flow, occurrence of gas hydrates, geochemistry, substratum and sediment stability. Carbonate precipitation, a by-product of microbial anaerobic hydrocarbon oxidation, and biogenic habitats created by foundation invertebrate species (symbiont bearing, sponges, soft and stony corals) provide substrata for a variety of macrofaunal

and meiofaunal assemblages. These faunal assemblages respond to changes in structural complexity, habitat geochemistry, nutrient sources, and biotic interactions. Diversity may be maintained by a combination of biogenic microhabitat heterogeneity, disturbance created by feeding activities of larger animals, and food resources (Grassle & Maciolek 1992)

In the Gulf of Cadiz the complex topographic, geological and hydrological settings produce a considerable environmental heterogeneity that is expressed by the occurrence of a rich and diverse range of habitats, comprising mud volcanoes, diapiric ridges, carbonate chimney fields, cold water coral reefs. As shown by our results the different habitats are inhabited by bivalve assemblages with differing taxonomical and trophic structures that likely respond to the variability in food sources either chemosynthetically or photosyntetichally derived, availability of substrates (such as carbonate concretions or cold-water stony corals) but also possibly to the hydrological regime of the different water masses.

Although changes in the depth zonation of bivalve assemblage are not so strong as those reported for other taxa, bathymetric discontinuities are often more or less clearly delimited (Allen & Sanders 1996; Olabarria 2005). Although the causes of zonation in the deep-sea area complex (e.g. Rex 1977), hydrographic conditions have been invoked as being the most important in areas of the NE Atlantic (Rice *et al.* 1990; Billett 1991; Howell *et al.* 2002). Also in the Gulf of Cadiz the main regions of enhanced turnover in the bivalve assemblage coincide with vertical oceanographic boundaries (Eastern North Atlantic Central Water, Mediterranean Water; North Atlantic Deep Water) and therefore the water mass structure and temperature may be hypothesised as also playing an important role in determining faunal zonation.

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Chapter 2.5.Deep-sea ophiuroids (Echinodermata) from mud volcanoes in the Gulf of Cadiz (NE Atlantic)

Abstract

The Ophiuroidea collected from mud volcanoes and adjacent bathyal environments from the Gulf of Cadiz are reviewed. Thirteen species included in four families, Ophiacanthidae, Ophiactidae, Amphiuridae and Ophiuridae were identified. A direct relationship to the chemosynthetic assemblages has not been established as the ophiuroids found in the mud volcanoes do not appear to have novel morphological adaptations and are also occurring in non reducing environments. The ophiuroid fauna from the Gulf of Cadiz differs from other cold seep regions not only by the high species richness but also because Amphiuridae are dominant both in number of species and abundance. One species previously unknown, *Ophiopristis cadiza* sp nov., (Ophiocanthidae) was collected from a dead cold-water coral thicket at the flank of a mud volcano and differs from its congeners in the type of disk spines which are more rugose and not smooth as in most of the other species, the presence of the thickened integument in larger specimens and the distinct separation between the oral papillae and the second oral tentacle scales.

Keywords: mud volcanoes, reducing environments, macrofauna, biodiversity

Rodrigues CF, Paterson G, Cabrinovic A & Cunha MR (accepted with revisions) Deep-sea ophiuroids (Echinodermata) from mud volcanoes in the Gulf of Cadiz (NE Atlantic). *Zootaxa*

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Deep-sea ophiuroids (Echinodermata) from mud volcanoes in the Gulf of Cadiz (NE Atlantic)

2.5.1.Introduction

The first deep-water ophiuroid was discovered more than 150 years ago in the North Atlantic at a depth of 1460m during the sounding of Baffin Bay (Müller & Troschel 1842 in Menzies *et al*. 1973). During late 19th and early 20th centuries, ophiuroid records have significantly increased due to the collection of many specimens by several deep-water expeditions (Martynov & Litvinova 2008). However, the most important studies on ophiuroids of the Atlantic have been carried out during the past 40 years (e.g. Cherbonnier & Sibuet 1972; Gage *et al*. 1983; Paterson 1985; Smith *et al*. 1985; Bartsch 1987, 1991; Stöhr & Segonzac 2005) and a taxonomic and biogeographical review has been carried out recently by Martynov and Litvinova (2008). These studies aimed to expand the global knowledge on this group but, despite this effort, the deep-sea ophiuroid fauna remains poorly known as many species are only reported from a single or a few individuals. Because morphological identification of ophiuroids is based almost exclusively on skeletal characters, which change greatly in number and shape during growth (Stöhr 2004, 2005), this lack of specimens is of great importance.

Ophiuroids, which have the highest abundances of all deep-sea megafauna taxa in non-reducing environments (Gage & Tyler 1991), have also been reported from several cold seeps and vents (Hecker 1985; Tyler *et al*. 1995; Sibuet & Olu 1998; Gebruk *et al*. 2003; Sahling *et al*. 2003; Van Dover *et al*. 2003; Stöhr & Segonzac 2005, 2006; Levin & Mendoza 2007). The unidentified status of several ophiuroid records suggests that the overall diversity of ophiuroids in these reduced environments remains underestimated. Seven species were reported from Blake Ridge, Gulf of Mexico and Barbados cold seeps in the Western Atlantic (Stöhr & Segonzac 2005), two from the Haakon Mosby mud volcano in the Eastern Atlantic (Gebruk *et al*. 2003) and two from the North Pacific Aleutian margin (Levin & Mendoza 2007). Presently, eight species have been reported from hydrothermal communities in the East Pacific

and Mid-Atlantic Ridge (MAR) (Stöhr & Segonzac 2005, 2006). From these, only one, *Ophienigma spinilimbatum* Stöhr & Segonzac 2005, is considered endemic to Western Atlantic cold seeps, one, *Ophioctenella acies*, Tyler *et al*., 1995, is considered endemic to reduced environments (present in Western Atlantic cold seeps and MAR vents), and two, *Spinophiura jolliveti* Stöhr & Segonzac, 2006, and *Ophiolamina eprae* Stöhr & Segonzac, 2006, are endemic to East Pacific vents (Stöhr & Segonzac 2005, 2006). Although some other ophiuroid species are only known from seeps and vents, their low abundance and rare occurrence suggest that they are not endemic to reducing habitats. Some of the non-endemics are possibly opportunistic species (that may reach relatively high densities) or accidental species (usually occurring at low densities) known from other habitats. For most of the ophiuroid species occurring in reducing habitats a direct relationship to the chemosynthetic assemblages has not been established and therefore they may have a broader distribution.

Information on the occurrence and distribution of ophiuroids from the Atlantic continental margins of Morocco and Iberian Peninsula was until now scarce. Marques (1980) and Bartsch (1987, 1991) recorded the distribution of *Amphipholis squamata* (Delle Chiaje, 1828), *Ophiactis lymani* Ljungman, 1871, *Ophiothrix fragilis* (Abildgaard, 1789) and *Ophiothrix indigna* Koehler, 1906 in the Southern Portuguese margin covering a bathymetric range from 500 to 820m. In this study we report on the Ophiuroidea recovered from mud volcanoes and adjacent environments, such as carbonate chimneys and cold-water scleractinean corals, from a depth range of 200 to 4000m in the Gulf of Cadiz. The aim of the paper is to extend the knowledge on the biodiversity and distribution of the ophiuroids in the Gulf of Cadiz, with particular emphasis on reducing habitats.

2.5.2.Material and methods

Study area. The present work is based on numerous samples from mud volcanoes in the Gulf of Cadiz located in the NE Atlantic, west of the Gibraltar Straits through which it connects to the Mediterranean Sea (Figure 2.5.1).

Mud volcanoes (MV) were firstly reported on the Moroccan margin by Gardner (2001) and the first evidence of seep-related fauna in the Gulf of Cadiz was briefly mentioned by Pinheiro *et al.* (2003) from samples collected in the Moroccan, Spanish and deep Portuguese margins. A brief overview of the hydrological, geological and biogeochemical settings of the Gulf of Cadiz is given by Rodrigues *et al*. (2008). The results reported herein refer to samples collected from exposed carbonate chimneys' fields in submarine channels and diapiric ridges associated to erosion effects of the Mediterranean Outflow Water, and from three main areas of mud volcanoes in the Gulf of Cadiz: the shallow El Arraiche Moroccan field, the Western Moroccan field and the deep Portuguese field.

The El Arraiche field encompasses Renard Ridge (including Pen Duick Escarpment), Vernadsky Ridge and several mud volcanoes located at depths from 200 to approximately 700m depth. Dead cold-water scleractinean coral reefs, carbonate crusts and exposed carbonate chimneys characterise the Renard and Vernadsky Ridges. Carbonate crusts, rock blocks and clasts are often found in the craters of the shallow mud volcanoes where mild seepage activity has been recorded (Van Rensbergen *et al*. 2005). The faunal assemblages are characterised by high species richness and abundance of background fauna (mostly peracaridean crustaceans and polychaetes) and the most common chemosynthetic species are solemyid bivalves (*Petrasma* sp.) and Siboglinid polychaetes (*Siboglinum* spp.). Mercator, one of the shallowest mud volcanoes differs significantly from the other mud volcanoes by the high chloride enrichment of its pore water. The top of the structure shows patches of disturbed sediments from which gas venting is occasionally observed (M.R. Cunha, pers. observation). Solitary corals (*Caryophyllia* sp.), accompanied by Cidaridae echinoids and Onuphidae polychaetes (*Hyalinoecia tubicola* (O. F. Mueller 1788)) are abundant at the central area of the crater.

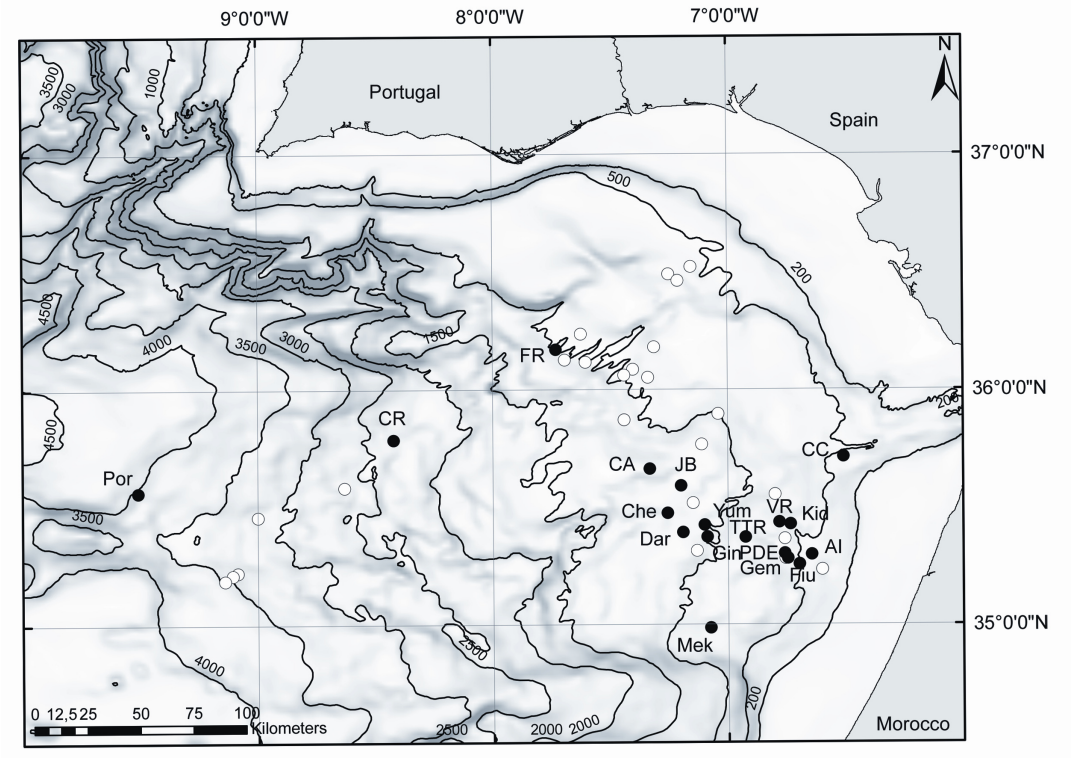

Figure 2.5.1. Map of the study area, the Gulf of Cadiz, and location of sampling sites. Full circles: sampling sites with Ophiuroidea; Empty circles: other sites; AI: Al Idrisi MV; CA: Captain Arutyunov MV; CC: carbonate chimneys West of Gibraltar Straits; CR: Carlos Ribeiro MV; Che: Chechaouen MV; Dar: Darwin MV; Fiu: Fiúza MV, FR: Formosa Ridge; Gin: Ginsburg MV; JB: Jesuz Baraza MV; Kid: Kidd MV; Mer: Mercator MV; Mek: Meknès MV; PDE: Pen Duick Escarpment; Por: Porto MV; TTR: TTR MV; VR: Vernadsky ridge; Yum: Yuma MV.

The western Moroccan field comprises several mud volcanoes at depths between 900 and 1200m where the occurrence of extensive *Neptunea* and *Bathymodiolus* graveyards and carbonate formations suggest that this was a very active seepage area in the past. Solemyid bivalves (*Acharax* sp.) and siboglinid polychaetes (*Siboglinum* spp.) are the most common chemosynthetic species. Darwin mud volcano differs from the others in this area because its crater is completely covered by large carbonate slabs and crusts; the fissures among slabs and depressions with scattered crust are filled with abundant shell ash and occasionally small clumps of living *Bathymodiolus mauritanicus* Cosel 2002.

Meknès is the southernmost Moroccan mud volcano rising isolated among an extensive field of small coral mounds. The crater is formed by stiff, sometimes heavily disturbed, green mud breccia with scattered clasts and a

striking large number of empty shells of the gastropod *Neptunea contraria* (M.R. Cunha, pers. observation). Living megafauna is rarely sighted and chemosynthetic fauna consists of *Acharax* sp and *Siboglinum* spp.

The deep-water field (1300-4000m), mostly within the Portuguese margin includes several mud volcanoes that are aligned along major crustal strike–slip faults associated with the African-Eurasian plate boundary (Duarte *et al*. 2005). Gas hydrates were recovered from these mud volcanoes and the methane concentrations yield the highest records from the Gulf of Cadiz. Chemosynthetic fauna is highly diverse and include siboglinid species of the genus *Siboglinum*, *Polybrachia*, *Lamellisabella*, *Spirobrachia* and *Bobmarleyia* (Hilário & Cunha 2008, Hilário *et al*. submitted), solemyid (*Acharax* sp.), thyasirid (Rodrigues *et al*. 2008) and vesicomyid bivalves. These mud volcanoes appear to show a high degree of endemism and low penetration of background fauna in the most active areas of the crater.

Collection and preparation of specimens. Macrofaunal samples were collected from mud volcanoes and adjacent habitats such as fields of carbonate chimneys and crusts and cold-water coral thickets (mostly dead) during several cruises carried out since 2000. Samples reported herein were taken from 19 sites during TTR11 to TTR16 cruises onboard the RV Prof. Logachev (IOC – MSU) and MSM01-03 cruise onboard the RV Maria S. Merian (IFM-GEOMAR) using mostly a TV-assisted grab or a USNEL box-corer. Occasionally faunal specimens were also recovered from Kasten-corer, multiple corer or lander samples that were carried out for different purposes. Whenever possible the specimens were sorted on board and preserved in 70 or 96% ethanol (the latter preserved for molecular analysis). Data on the stations yielding ophiuroids are presented in (Table 2.5.1). Al Idrisi, Mercator, Fiuza, Kidd, and TTR mud volcanoes are included in the El Arraiche field, Yuma, Ginsburg, Jesuz Baraza, Darwin and Chechaouen in the Western Moroccan field and Captain Arutyunov, Carlos Ribeiro and Porto in the deep-water field.

Type specimens have been deposited at Natural History Museum and the remaining material is deposited in the Biological Research Collection of the Department of Biology, University of Aveiro.

The taxonomic classification follows the scheme suggested by Smith *et al*. (1985).

Abbreviations

DBUA, Department of Biology, University of Aveiro (Biological Research Collection); IFM-GEOMAR, Institut für Meereskunde - Forschungszentrum für marine Geowissenschaften; IOC-UNESCO, Intergovernmental Oceanographic Commission – United Nations Educational, Scientific and Cultural Organization; MSU: Moscow State University; NHM, Natural History Museum; NOCS: National Oceanographic Center, Southampton; TTR, training Through Research programme.

2.5.3.Systematics

Order Ophiurida Müller & Troschel, 1840

Suborder Ophiurae Müller & Troschel, 1842

Family Ophiacanthidae Perrier, 1891

SubFamily Ophiotominae Paterson 1985

***Ophiopristis cadiza* sp nov.** (Figure 2.5.2, Figure 2.5.3)

Material examined. *Holotype:* NE Atlantic: Gulf of Cadiz, Captain Arutyunov MV, MSM01-03, stn 195, one specimen (NHM XXXX).
Paratypes: same data as holotype, twenty specimens (DBUA XXXX).

Description. Disk is sub-pentagonal to round, disk diameter 3.5 to 10 mm. Arms at least four times disk diameter. Disk covered with thin scales, many carrying a spinelet. Spinlets long, rugose, tip of spinlet with two or three small pointed rugosities; spinelets in middle of disk finer than those on the periphery or in the interbranchial region. Radial shields exposed, approximate triangular in shape; each shield pair separated along whole length; length extending slightly less than a quarter of the disk diameter. Ventral interbranchial area covered with small scales. Spinelets present but shorter and with a lower density than aboral surface.

Oral surface in larger specimens covered by thin skin, partially obscuring the plates beneath in larger specimens. Jaws slightly longer than broad. Two apical papillae on most jaws. Up to five oral papillae edge jaw, small pointed, distal papillae lie deeper within mouth. Distinct separation between oral papillae and tentacle scales associated with the second oral tentacle pore. Oral tentacle scales larger than oral papillae, four to five in number. Radial shields pentagonal slightly longer than broad, in larger specimens, slightly more elongated and rounded in smaller specimens. Adoral shields large and separating oral shields from first lateral arm plate.

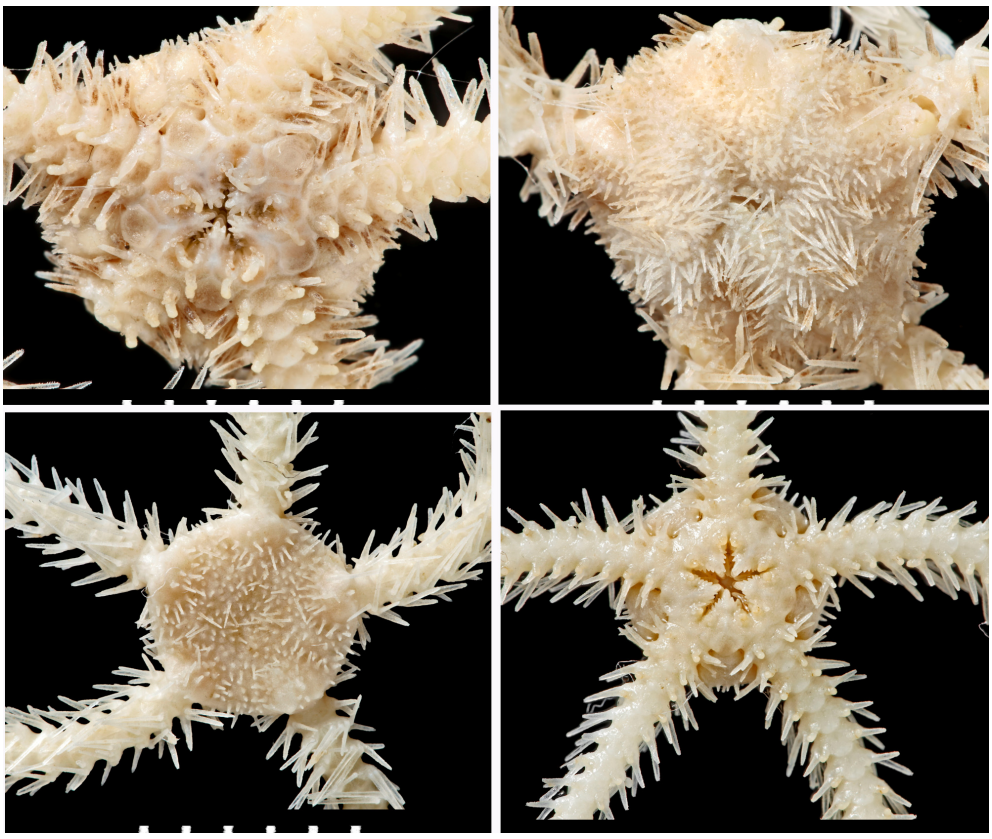

Figure 2.5.2. *Ophiopristis cadiza* sp. nov. a) and b) Holotype large specimen, c) and d) smaller specimen, one of the paratypes. Scale bars = 1 cm.

Arms long distinctly nodded in proximal segments, arm plates becoming glassy. Dorsal arm plates triangular to scallop-shaped with curved distal edge; plates contiguous at least on proximal segments; on distal segments dorsal arm plates more triangular and just contiguous. Ventral arm plates approximately rectangular with a curved distal edge, contiguous on proximal arm segments. Tentacle pores covered by large, flat tentacle scales, in the

proximal segments each pore with three to four scales arranged around the pore, reducing to two in more distal segments, two scales comprised of a large slightly pointed, almost leaf-like scale sitting over a smaller inner scale. Lateral arm plates flared forming a ridge along the area where arm spines inserted. Arms spines 6, long up to five segments in length; not quite forming a continuous row in the first free arm segment. Dorsalmost spines finely rugose, slightly flattened; ventralmost spines shorter with rugose edge. These spines become more developed on one edge, forming a 'hook'-like spine.

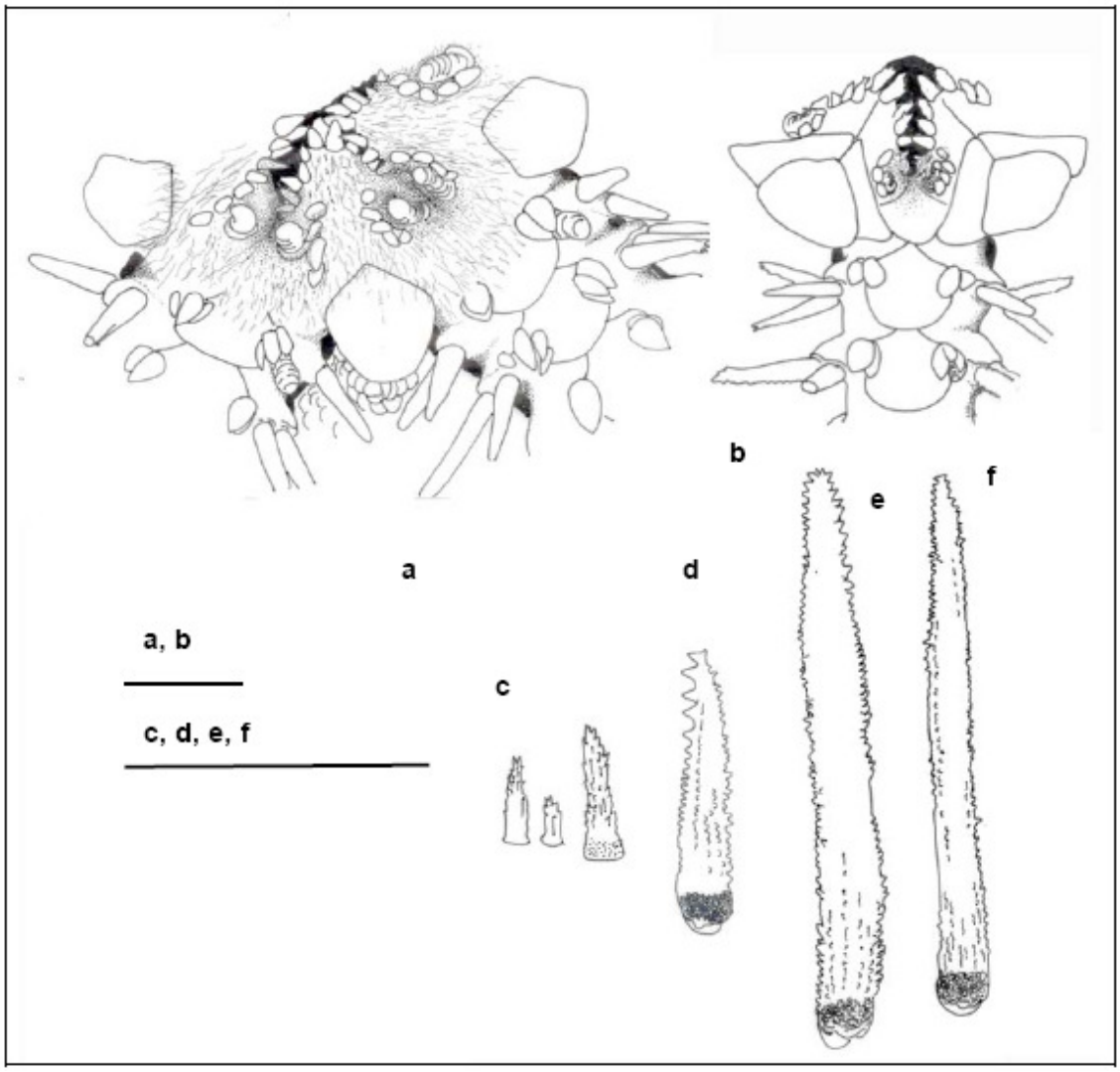

Figure 2.5.3. *Ophiopristis cadiza* sp. nov. a) oral view of adult (Disk diameter: 9.5 mm), b) oral frame of juvenile specimen (disk diameter: 3.5 mm), c) disk spinlets, d) ventralmost spine from distal arm segment, e) mid arm spine f) dorsalmost arm spine. Bar scales = 1 mm.

Distribution and ecology. This species is only known from the type locality. It was collected from a coral thicket (*Dendrophylia* cf. *alternata*, mostly dead

branches with a few living polyps) in a single station at the flanks of Captain Arutyunov MV together with *Ophiactis abyssicola*, *Ophiacantha aristata* and *Amphioplus hexabrachiatus*. Known bathymetric range: 1390m.

Etymology. This species is named after the area (Gulf of Cadiz) where it was first discovered.

Remarks. Placing this species within *Ophiopristis* Verril, 1899 represents a compromise and potentially expands the definition of the genus. There are a number of characters which appear to be unique. The emergent radial shields is unusual in the *Ophiotominae* and in *Ophiopristis*, the arrangement of the oral papillae and second oral tentacle scales are also distinct and finally the development of thickened integument has not been reported within species of the genus. It is possible that this combination of characters within this species represents a new genus, however as O'Hara & Stöhr (2006) and Paterson (1985) point out, the genera within Ophiacanthidae are not well defined and so adding another would only serve to add to this problem. Ophiopristis cadiza share the following features with other species of the genus – the arms spines are slightly flattened with a rugose edge, the oral and second tentacle papillae are separated either physically or by shape and size, the tentacle scales are multiple in proximal arm segments.

Ophiopristis can now be defined as: jaws longer than broad, flanked on each side by four or more oral papillae, second oral tentacle pore superficial opening near the surface of the jaw, armed with tentacle scales similar in size and shape to the oral papillae, sometimes confluent with oral papillae but in some separated; adoral shields long narrow separating the oral shield from the first lateral arm plate; disk covered with small scales carrying a elongated spinelet, often long, hollow and with pointed or furcate tips; radial shield usually concealed but maybe exposed; arms spines flattened carrying a row of sharp points along each edge; tentacle pores armed with up to four tentacle scales on proximal pores reducing to one to two large scales on distal segments.

According to Stöhr & O'Hara (2007) there are 10 species of *Ophiopristis*. *O. cadiza* differs in the type of disk spines which are more rugose and not smooth as in most of the other species; the presence in larger specimens of the

thickened integument and the distinct separation between the oral papillae and the second oral tentacle scales. *O. cadiza* is similar to *O. dissidens* Koehler, 1905 in having three tentacle scales on proximal tentacle pores but differs in that the disk spinelets are not smooth, glassy and hollow.

Variation. Smaller individuals do not have the thickened skin over the oral surface so the plates are clearer (Figure 2.5.3). Here the oral shield is more rounded slightly wider than long. The oral papillae are slightly more rugose than in larger specimens. However in other characters, oral papillae and tentacle scale number and arrangement, smaller specimens resemble the larger.

SubFamily Ophiacanthinae Paterson 1985

Ophiacantha aristata Koehler 1896

Material examined. Captain Arutyunov MV, MSM01-03, stn 195, one specimen. (DBUA.001063.01).

Remarks. This species presents a round disk, covered with spinelets. These are crowned with 9 or more points which may be subdivided into secondary points giving the crown a very irregular appearance. The jaws are as broad as long. The arms are distinctly nodded and the arm spines are highly rugose and the tentacle scale is also rugose and spine-like (Paterson 1985).

Ecology and distribution: This is a bathyal North Atlantic species (822-1700m) known from SW Ireland south to the Canary Islands (Paterson 1985). In the Gulf of Cadiz it was collected from a coral thicket (*Dendrophylia* cf. *alternata*, mostly dead branches with a few living polyps) in a single station at the flanks of Captain Arutyunov MV (1390m) together with *Ophiopristis cadiza*, *Ophiactis abyssicola* and *Amphioplus hexabrachiatus*.

Family Ophiactidae Matsumoto, 1915

Ophiactis abyssicola (M. Sars, 1861)

Material examined. Captain Arutyunov MV, MSM01-03, stn 195, three specimens (DBUA.001064.01).

Remarks. The disk may be round or indented, covered by large plates amongst which the centrodorsal plate and usually the primary plates are distinct. There are often conical spinelets on both the dorsal and the ventral interradial plates, that can be absent from one or other side or even completely missing. There is a tricuspid or heart shaped apical papilla flanked on each side by two distal oral papillae, which arise on the adoral shields; the dorsal arm plates are fan shaped and contiguous at least proximally. The ventral arm plates are pentagonal (Paterson 1985)

Ecology and distribution: This is an Atlantic species known from a wide bathymetric range (125-4721m) from Norway to southern Africa (Paterson 1985). In the Gulf of Cadiz it was collected from a coral thicket (*Dendrophylia* cf. *alternata*, mostly dead branches with a few living polyps) in a single station at the flanks of Cap. Arutyunov MV (1390m) together with *Ophiopristis cadiza*, *Ophiacantha aristata* and *Amphioplus hexabrachiatus*.

Ophiactis balli (Thompson, 1840)

Material examined. Al Idrisi MV, TTR12, stn AT412, one specimen (DBUA.001065.01); West of Gibraltar Straits, TTR14, stn AT550, one specimen (DBUA.001066.01).

Remarks. The disk is round, covered with small plates amongst which the primary plates are inconspicuous; there are often small spinelets on the ventral side on the edge of the disk; there is a pointed or heart shaped apical papilla with one pointed oral papilla on each side usually arising on the oral plates; the ventral arm plates are almost square; there are 5 conical arm spines proximally, the middle ones usually the largest, distally there are only 4 (Paterson 1985)

Ecology and distribution. This is an Eastern Atlantic species known from depths of 60-400m (Paterson 1985), generally found in crevices or amongst sessile invertebrates with the body concealed and the arms extended out into the water. It is characteristic of exposed or moderately exposed rocky situations especially on limestone (Picton & Morrow 2007). In the Gulf of Cadiz it was collected within its known bathymetric range from limestones at the

crater of Al Idrisi MV and from carbonate chimneys in an exposed site (high bottom currents) West of Gibraltar Straits.

Family Amphiuridae Ljungman, 1867
SubFamily Amphiurinae Matsumoto, 1915

Amphiura grandisquama Lyman, 1869

Material examined. Vernadsky Ridge, TTR15, stn AT574, one specimen (DBUA.001067.01); Pen Duick Escarpment, TTR16, stn AT600, seven specimens (DBUA.001068.01).

Remarks. The disk is round, indented over the arms, covered with small scales; the ventral interradial areas are covered by small scales; this species is characterized by the long ventralmost arm spine, the shape of the distal oral papillae and having one tentacle scale on each pore (Paterson 1985).

Ecology and distribution: In the Eastern Atlantic *A. grandisquama* has been recorded from Iceland south to the Azores and Cape Verde Islands at depths of 861-1635m (Paterson 1985). In the Gulf of Cadiz (Figure 2.5.4A) it was collected from carbonate chimneys and crusts at shallower depths (508-610m).

Amphiura chiajei Forbes, 1843

Material examined. Mercator MV, TTR15, stn AT569, one specimen (DBUA.001069.01), stn AT576, 15 inds. (DBUA.001069.02), stn AT577, two specimens. (DBUA.001069.03); Kidd MV, TTR14, stn AT560, one specimen (DBUA.001070.01).

Remarks. The disk is covered with scales which become smaller towards edge; there are up to 8 straight pointed arm spines (Paterson 1985).

Ecology and distribution: *Amphiura chiajei* has been recorded from the eastern Atlantic and Mediterranean, and from West Africa; it is usually a shelf species but has been recorded from a depth of 1200m (Paterson 1985). This

species is reported as living buried in muddy sand, extending its arms above the surface of the sediments to feed on deposited material. In the Gulf of Cadiz (Figure 2.5.4A) it was collected from soft mud breccia in the crater of Mercator and Kidd mud volcanoes within its known bathymetric range.

Amphiura filiformis (O.F. Müller, 1776)

Material examined. Al Idrisi MV, TTR12, stn AT412, one specimen. (DBUA.001071.01); Mercator MV, TTR15, stn AT569, nine specimens (DBUA.001072.01); stn AT575, 24 specimens. (DBUA.001072.02); stn AT576, nine specimens (DBUA.001072.03); MSM01-03, stn 237, two specimens (DBUA.001073.01); stn 241, 32 specimens (DBUA.001073.02); stn 242, 24 specimens (DBUA.001073.03); stn 267, two specimens. (DBUA.001073.04); stn 287,three specimens (DBUA.001073.05); Fiuza MV, TTR14, stn AT566, five specimens (DBUA.001074.01); West of Gibraltar Straits, TTR14, stn AT552, one specimen (DBUA.001074.02); Pen Duick Escarpment, TTR12, stn AT407, one specimen (DBUA.001071.02); TTR16, stn AT602, one specimen (DBUA.001075.01).

Remarks. The disk is rounded to subpentagonal; the centradorsal and primary plates are conspicuous; the first oral tentacle scales are not very calcified and have a membranous appearance; they are usually 5-7 arms spines proximally. The second, often third and fourth have a compressed axe-shaped tip (Paterson 1985).

Ecology and distribution: *Amphiura filiformis* is a common, predominantly shallow-water species of the north-eastern Atlantic (Paterson 1985). It is known to occur from Norway to West Africa and also from the Mediterranean Sea at depths up to 1665 m. This species is often found sympatric with *A. chiajei* and accurate descriptions of all growth stages of both species are therefore desirable (Stöhr 2005). Both species live buried in fine muddy sand but, unlike its congener, *A. filiformis* feeds into the water current extending its arms vertically 3-4cm above the sediment surface (Vopel *et al*. 2003). In the Gulf of Cadiz (Figure 2.5.4B) *A. filiformis* co-occured with *A. chiajei* in Mercator MV but reached the highest density (107ind.m^{-2}) in the crater where only one

specimen of *A. chiajei* was collected whereas the latter reaches densities of 60 ind.m^{-2} in the flank where the density of *A. filiformis* drops to 36 ind.m^{-2}. *Amphiura filiformis* has also a much wider distribution and has been recorded in two other mud volcanoes (Al Idrisi and Fíuza) and in association to carbonate chimneys and crusts at two other sites (West of Gibraltar and Pen Duick Escarpment) at depths between 230 and 556m.

Amphiura sp. sensu Stöhr and Segonzac, 2005

Amphiura sp. Stöhr and Segonzac 2005

Material examined. Al Idrisi MV, TTR12, stn AT412, one specimen (DBUA.001076.01); Kidd MV, TTR14, stn AT528, nine specimens (DBUA.001077.01); stn AT560, one specimen (DBUA.001077.02); Meknès MV, TTR14 stn AT541, four specimens (DBUA.001077.03); Yuma MV, TTR16, stn AT604, one specimen (DBUA 001078.01); Darwin MV, TTR16, stn AT608, one specimen (DBUA.001078.02); Captain Arutyunov MV, TTR14, stn AT546, three specimens (DBUA.001077.04); West of Gibraltar Straits, TTR14, stn AT551, one specimen (DBUA.001077.05); Pen Duick Escarpment, TTR16, stn AT600, one specimen (DBUA.001078.03).

Remarks. This species is the same as recorded by Stöhr and Segonzac (2005) from West Atlantic cold seeps. The species is characterised by just three arm spines, a character shared with other *Amphiura* species.

Ecology and distribution: A single specimen of *Amphiura* sp. sensu Stöhr & Segonzac was previously known from mud sediments among chemosynthetic fauna (*Bathymodiolus boomerang*, *Escarpia* sp.) of the Orinoco cold seeps, West Atlantic, at 1947m. In the Gulf of Cadiz (Figure 2.5.4C) this species of *Amphiura* has a wide distribution in the shallow and western areas of the Moroccan margin (carbonate provinces) predominantly associated to the presence of carbonate crusts and covering a bathymetric range of. 230-1345m. It was recorded from six mud volcanoes and two other sites with carbonate chimneys or crusts, often co-occurring with Amphipholis squamata and occasionally with other Amphiuridae (*Amphioplus hexabrachiatus*, *Amphiura filiformis* and *Amphiura grandisquama*).

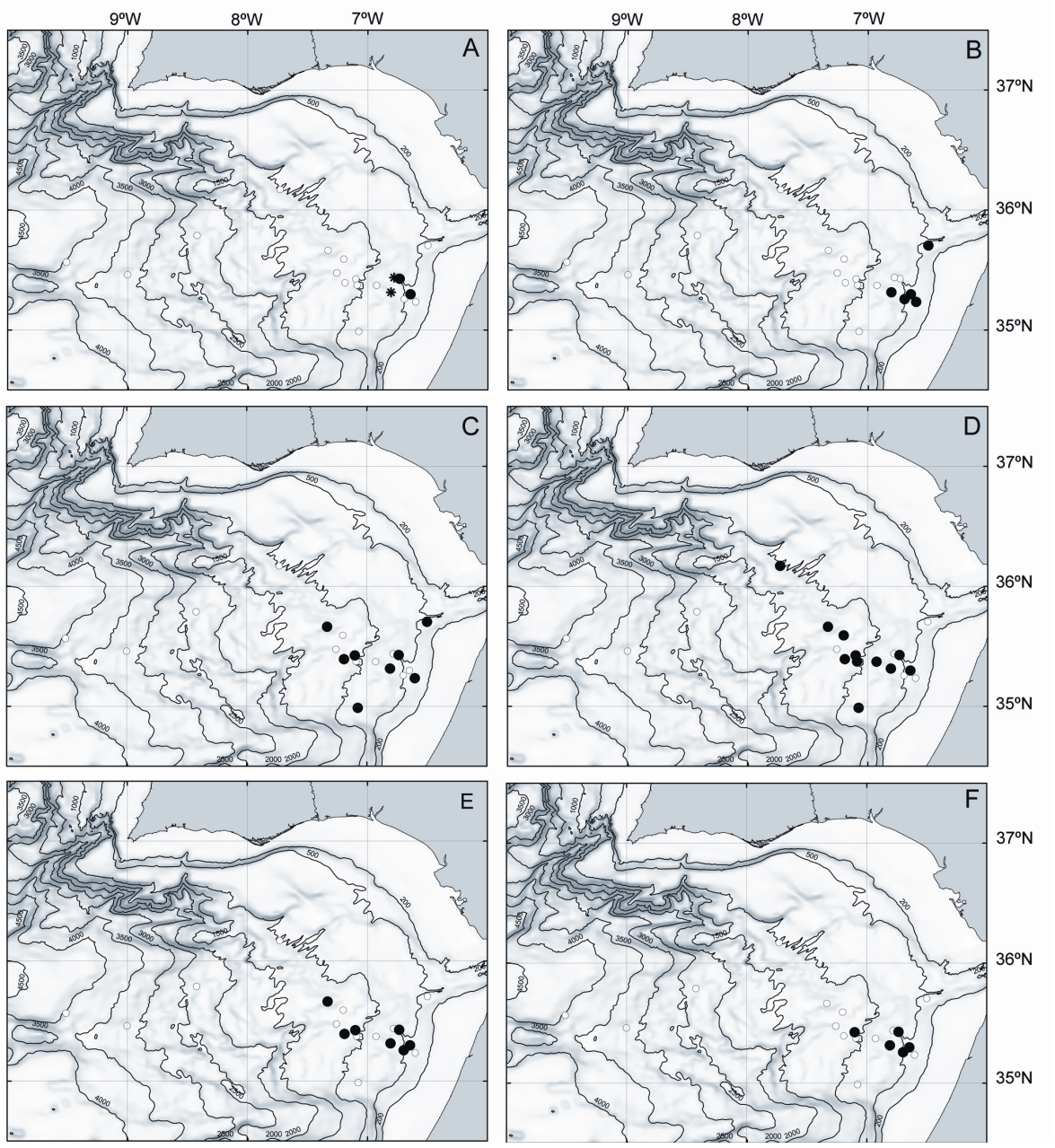

Figure 2.5.4. Distribution of Amphiuridae species in the Gulf of Cadiz. A: *Amphiura grandisquama* (full circles) and *Amphiura chiajei* (asterisks); B: *Amphiura filiformis*; C: *Amphiura* sp. sensu Stöhr and Segonzac; D: *Amphipholis squamata*; E: *Amphioplus hexabrachiatus*; F: *Amphilepis ingolfiana*.

Amphipholis squamata (Delle Chiaje, 1829)

Material examined. Mercator MV, TTR14, stn AT568, one specimen (DBUA.001079.01); TTR15, stn AT575, 10 specimens (DBUA.001080.01), stn AT576, five specimens (DBUA.001080.02), stn AT577, two specimens (DBUA.001080.03); MSM01-03, stn 241, 14 specimens (DBUA.001081.01),

stn 242, seven specimens (DBUA.001081.02), stn 287, one specimen (DBUA.001081.03); Fiuza MV, TTR14, stn AT566, two specimens (DBUA.001079.02); Kidd MV, TTR14 stn AT559, one specimen (DBUA.001079.03), stn AT560, nine specimens (DBUA.001079.04), stn AT561, three specimens (DBUA.001079.05); TTR MV, TTR12, stn AT416, 16 specimens (DBUA.001082.01); Meknès MV, TTR14, stn AT541, one specimen. (DBUA.001079.06); Meknès MV, TTR15, stn AT581, one specimen (DBUA.001080.04), stn AT585, one specimen (DBUA.001080.05), stn AT587G, one specimen (DBUA.001080.06); MSM01-03, stn 321, seven specimens (DBUA.001081.04), stn 335, 15 specimens (DBUA.001081.05); Yuma MV, TTR16, stn AT604, three specimens (DBUA.001083.01); Ginsburg MV, TTR16, stn AT607, three specimens (DBUA.001083.02); Jesuz Baraza MV, TTR12, stn AT391, one specimen (DBUA.001082.02); Darwin MV, TTR16, stn AT608, eight specimens (DBUA.001083.03); Captain Arutyunov MV, TTR12, stn AT399, one specimen (DBUA.001082.03); MSM01-03, stn 182, one specimen (DBUA.001081.06), stn194, two specimens (DBUA.001081.07), stn 223, four specimens (DBUA.001081.08), stn 246, four specimens (DBUA.001081.09), stn 297, one specimen (DBUA.001081.10); Formosa Ridge, TTR12, stn AT388, one specimen (DBUA.001082.04), stn AT389, five specimens (DBUA.001082.05); West of Gilbratar Straits, TTR14, stn AT550, six specimens (DBUA.001079.07), stn AT552, 24 specimens (DBUA.001079.08); Pen Duick Escarpment, TTR12, stn AT406, 18 specimens (DBUA.001082.06); TTR14, stn AT565, one specimen (DBUA. 001079.09); TTR16, stn AT600, eight specimens (DBUA.001083.04), stn AT602, four specimens (DBUA.001083.05);

Remarks. The disk is round covered by coarse scales amongst which the centrodorsal and primary plates are indistinguishable except in small individuals. The ventral interradial plates are slightly smaller than the dorsal plates and there is a distinct boundary between them (Paterson 1985).

Ecology and distribution: *Amphipholis squamata* is known from several substrata (under stones, amongst algal and bryozoan turfs, rockpool weeds and occasionally on sandy bottoms) from the intertidal to 1200m. It is a cosmopolitan species abundant in the Eastern Atlantic from Iceland along the

European margin, Mediterranean Sea, West coast of Africa, west and east coast of South Africa and Madagascar (Alva & Vadon 1989). This species can switch from deposit feeding by collecting particles within its tube feet to suspension feeding via trapping detritus in mucus. It is hermaphroditic and the eggs are brooded and hatched as juveniles (Rowley 2006). In the Gulf of Cadiz (Figure 2.5.4D) it was the most common ophiuroid, recorded from 10 mud volcanoes and at three other sites in association to carbonate chimneys and crusts as well as cold-water corals. Young, intermediate and adult stages were found together at the same localities. This species was found at depths from 350 to 1379m which is its deepest record known.

Amphioplus hexabrachiatus Stöhr, 2003 (Figure 2.5.5)

Material examined. Mercator MV, TTR15, stn AT576, four specimens (DBUA.001084.01); Fiuza MV, TTR14, stn AT566, two specimens (DBUA.001085.01); Kidd MV, TTR14, stn AT559, two specimens (DBUA.001085.02); Yuma MV, TTR14, stn AT524, one specimen (DBUA.001085.03); Darwin MV, TTR16, stn AT608, one specimen (DBUA.001086.01); Captain Arutyunov MV, MSM01-03, stn 195, one specimen (DBUA.0010.87.01); Pen Duick Escarpment, TTR16, stn AT600, one specimen (DBUA.001086.02).

Remarks. This species is hexamerous, reproduces asexually by division, and reaches a maximum size of about 2.5 mm disk diameter. This is the first six-armed species of Amphioplus and the third species of the genus known from the North Atlantic. The specimens from the Gulf of Cadiz closely resemble the description and images given by Stöhr (2003). This is obviously a morphologically variable species; in the range of body sizes observed in this study, key features such as the arrangement of the oral papillae and buccal scale are often not developed. Similarly, it is only in the larger specimens (disk diameter > 2.00mm) that the tentacle scales develop and are conspicuous (Figure 2.5.5). On the dorsal surface the development of the radial shields changes from small plates only separated at the inner edge to more elongated, larger plates separated nearly the full length of the plate only

contiguous at the outer end (Figure 2.5.5). Smaller specimens show distinct evidence of fissiparity with one side of the disk being less well developed and smaller.

Ecology and distribution: This species was described from southwest of Iceland at depths of 1000-1500m, collected by the Benthic Invertebrates of Icelandic Waters (BIOICE) programme (Stöhr 2003). In the Gulf of Cadiz (Figure 2.5.4E) it was collected from the Pen Duick Escarpment and mud volcanoes of the Moroccan margin usually in areas with carbonate crusts or coral framework at depths between 414 and 1390m extending the upper bathyal limit from 1000 m to 414m. The records from Cadiz also extend considerably the known geographical range of this species.

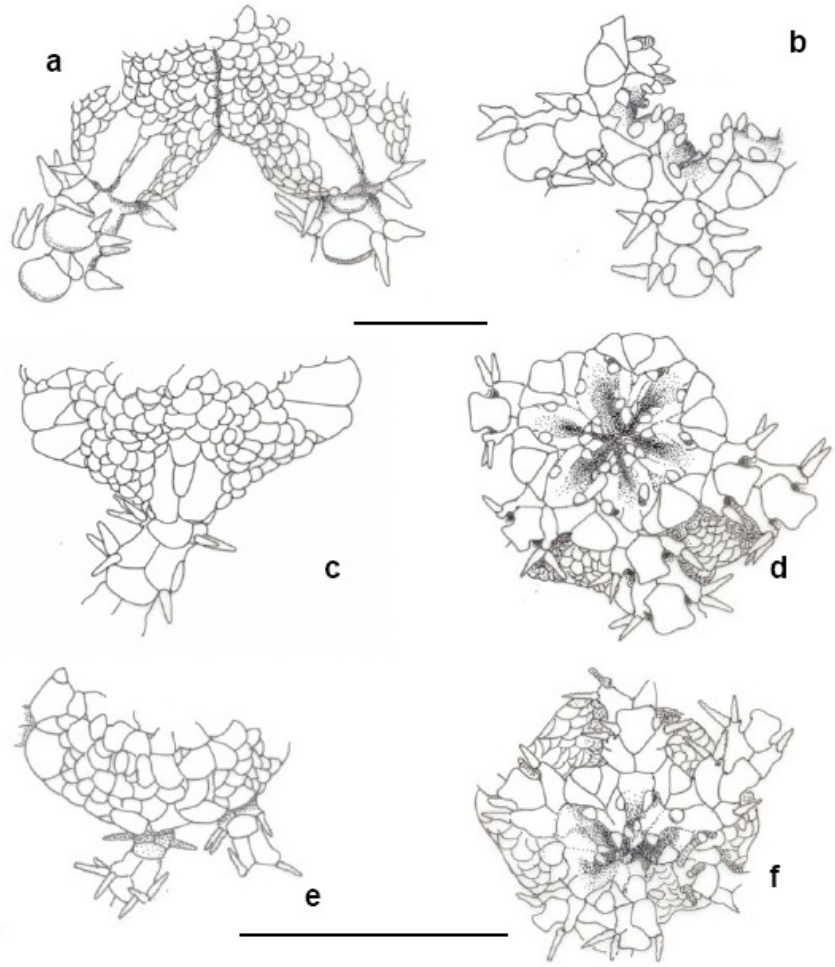

Figure 2.5.5. *Amphioplus hexabrachiatus*. Three specimens showing variation in both oral and aboral structures. a) and b) represent the largest diameter specimens with indented disk and well defined arrangement of oral papillae; c) and d) mid sized specimen; e) small specimen showing distinct signs of fissiparity. Bar scales = 1mm.

SubFamily Amphilepidinae Mortensen, 1933

Amphilepis ingolfiana Mortensen, 1933

Material examined. Mercator MV, TTR15, stn AT569, one specimen (DBUA.001088.01), stn AT575, two specimens (DBUA.001088.02), stn AT576, five specimens (DBUA.001088.03); MSM01-03, stn 237, one specimen (DBUA.001089.01); .Fiuza MV, TTR12, stn AT403, one specimen (DBUA.001090.01); Kidd MV, TTR14, stn AT559, two specimens (DBUA.001091.01); Yuma MV, TTR16, stn AT605, one specimen (DBUA.001092.01); Pen Duick Escarpment, TTR12, stn AT406, one specimen (DBUA.001090.02); TTR16, stn AT602, one specimen (DBUA.001092.02).

Remarks. This species can be distinguished because of the superficial second oral tentacle pore, the shape of the genital plate, by the scaling of the disk, the arrangement of the oral plates, the lack of tentacle scales and the erect arm spines (Paterson 1985).

Ecology and distribution: *Amphilepis ingolfiana* is a deep-sea species (957-4829m) known to occur in the NE Atlantic from the Rockall Trough south to off North Africa (Paterson 1985). In the Gulf of Cadiz (Figure 2.5.4F) it was collected at the Moroccan margin in four mud volcanoes and in the Pen Duick Escarpment, often associated with carbonate crusts and at shallower depths (353-975m) than previously reported.

Family Ophiuridae Lyman, 1865

SubFamily Ophiurinae Lyman, 1865

Ophiura (Dictenophiura) carnea Lütken, 1858

Material examined. Mercator MV, TTR15, stn AT569, one specimen (DBUA.001093.01).

Remarks. The disk is round to subpentagonal, high with almost vertical sides, covered with many plates amongst which centrodorsal and primary plates are usually distinct; there is one pointed apical papilla flanked by one spine-like and two block-like oral papillae (Paterson 1985).

Ecology and distribution. *Ophiura carnea* is reported from the Mediterranean Sea and in the Atlantic from Norway south to the Cape Verde Islands at depths ranging 40 to 2857m (Paterson 1985). In the Gulf of Cadiz it was recorded at a single station located near an active seepage area in the crater of Mercator MV co-occurring with *Amphiura chiajei*, *Amphiura filiformis* and *Amphilepis ingolfiana*.

SubFamily Ophiolepidinae Ljungman, 1867

Ophiomusium lymani W. Thomson 1873

Material examined. Porto MV, MSM01-03, stn 151, one specimen (DBUA.001094.01).

Remarks. This is a very common and easily identified member of the abyssal fauna; the disk is pentagonal, high, covered with relatively small plates amongst which usually only the centro-dorsal plate is distinct; there is a rudimentary arm comb of small, rounded, irregularly arranged papillae (Paterson 1985).

Ecology and distribution. *Ophiomusium lymani* is a bathyal and abyssal species with a wide distribution in the Atlantic and Indo-Pacific oceans (Alva & Vadon 1989). In the Atlantic it has been reported from SE Iceland south to Cap Blanc at depths ranging from 651 to 4829m (Paterson 1985). The single record from the Gulf of Cadiz is within its known bathymetric range. Only one specimen was collected from Porto MV among the frenulate (Polychaeta: Siboglinidae) fields that cover the crater.

2.5.4.Discussion

The Gulf of Cadiz is presently the most extensive cold seepage area known from the European margins spreading between 200 and 4000m depths and including over 40 mud volcanoes with a variable seepage activity. To date, processed biological samples are available from 18 mud volcanoes, from which 15 revealed ophiuroid occurrence (Table 2.5.1). In addition ophiuroids have

also been found in four sites from other bathyal environments such as cold-water coral thickets and fields of carbonate chimneys and crusts (Table 2.5.1).

A total of 434 individuals in 52 samples were examined and ascribed to thirteen different species, one of which, *Ophiopristis cadiza* sp. nov., previously unknown. Mercator was the mud volcano with higher abundance (up to 192 ind.m^{-2}) and species richness; a total of six species, from which up to five co-occurred in a single box-core sample (A=0.25m^2). Most samples yielded one to three species of ophiuroids and, in general, the shallower sites (<1400 m), which were also the best sampled, showed the highest species richness and abundance (Figure 2.5.6). Globally, low abundance of ophiuroids is reported from cold seeps (Stöhr & Segonzac 2005) but numbers per sample or area sampled are usually not available. The deeper mud volcanoes in the Gulf of Cadiz are also the most active and the low number of species recorded at these deeper sites maybe following a putative global pattern of low ophiuroid diversity at cold seeps. However, rather abundant, large-sized ophiuroids were often recorded during deep-towed TV observations in Porto (3800m: TTR16, TTR17, MSM01-03), Bonjardim (3100m: MSM01-03, TTR17), Olenin (2610m: TTR15) and Carlos Ribeiro mud volcanoes (2200m: TTR11, TTR16, MSM01-03) (M.R. Cunha, pers. observation). The low abundance estimates in the present study may be a consequence of undersampling – only a few samples were collected in the deeper mud volcanoes - or a result of the type of sampling gear - large ophiuroids tend to be dispersed as corer samplers approach the seafloor and thus these are not a good collecting gear for these animals.

The ophiuroids from Gulf of Cadiz were recorded from the shallowest (Al Idrisi, 230m) to the deepest (Porto, 3862m) mud volcanoes in this region and extend the known bathymetric ranges of *Amphilepis ingolfiana*, *Amphiura* sp. sensu Stöhr and Segonzac, *Amphiura grandisquama* and *Amphioplus hexabrachiatus*, to shallower depths and of Amphipholis squamata to greater depths (Figure 2.5.4, Figure 2.5.7).

Table 2.5.1. Station data for the Gulf of Cadiz samples yielding ophiuroids. Observations from TTR12 to TTR16 and MSM01-03 cruise reports (Kenyon *et al*. 2003,2006; Pfannkuche 2006; Akhmetzhanov *et al.* 2007, 2008). D: Dredge; G: gravity core; Gr: TV-assisted grab; UB: USNEL box core; MC: Multi core; K: Kasten core; BL: BIGO lander; FL: FLUFO lander.

Structure	Cruise	Station	Gear	Date (dd.mm.yy)	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
Mud volcanoes									
Al Idrisi	TTR12	AT412	D	17.07.2002	35º14.193′	06º36.609′	230	*O. balli; Amphiura* und; *A. filiformis*	Crater; limestones
Mercator	TTR15	AT568	G	25.07.2005	35º17,684′	06º39.131′	418	*A. squamata*	Flank; mud breccia
		AT569	Gr	25.07.2005	35º17.917′	06º38.717′	358	*O. carnea; A. chiajei; A. filiformis; A. ingolfiana*	Crater; mud breccia, near seep
		AT575	UB	26.07.2005	35º17.903′	06º38.715′	355	*A. filiformis;A. squamata; A. ingolfiana*	Crater; mud breccia; near seep
		AT576	UB	26.07.2005	35º17.657′	06º39.129′	428	Amphiuridae und.; *Amphiura* und; *A. chiajei; A. filiformis; Amphiura hexabrachiatus; A. squamata; A. ingolfiana*	Flank; mud breccia
		AT577	UB	26.07.2005	35º17.305′	06º39.672′	485	Amphiuridae und.; *A. chiajei; A. squamata*	Off MV; hemipelagic sediments
	MSM01-03	237	MC	06.05.2006	35º17.914′	06º38.687′	353	Amphiuridae und.; *A. filiformis; A. ingolfiana;* Ophiuroidea juv.	Crater; mud breccia, near seep
		241	UB	06.05.2006	35º17.918′	06º38.717′	353	*Amphiura* und.; *A. filiformis; A. squamata;* Ophiuroidea und.	Crater; mud breccia, near seep; *Caryophyllia* facies
		242	UB	06.05.2006	35º17.870′	06º38.810′	350	*A. filiformis; A. squamata*	Crater; mud breccia *Hyalonecia* facies
		267	MC	09.05.2006	35º17.875′	06º38.789′	350	*A. filiformis;* Ophiuroidea juv.	Crater; mud breccia *Hyalonecia* facies
		287	MC	11.05.2006	35º17.890′	06º39.059′	379	*A. filiformis; A. squamata;* Ophiuroidea juv.	Crater rim; mud breccia
Fiúza	TTR12	AT403	G					*A. ingolfiana*	Crater; mud breccia
	TTR14	AT566	Gr	09.08.2004	35º15.510′	06º41.702′	414	Amphiuridae und.; *Amphiura hexabrachiatus*; *A. filiformis; A. squamata*	Crater; mud breccia, covered by marl
Kidd	TTR14	AT528	Gr	03.08.2004	35º25.304′	06º43.972′	489	*Amphiura* und.; *Amphiura* sensu Stöhr & Segonzac	Crater; mud breccia
		AT559	UB	08.08.2004	35º24.777′	06º43.782′	552	*Amphiura hexabrachiatus*; *A. squamata; A. ingolfiana*	Off MV; hemipelagic sediments
		AT560	UB	08.08.2004	35º25.306′	06º43.976′	498	Amphiuridae und.; *A. chiajei; Amphiura* sensu Stöhr & Segonzac; *A. squamata*	Crater; mud breccia

Structure	Cruise	Station	Gear	Date (dd.mm.yy)	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
		AT561	UB	08.08.2004	35º25.602′	06º44.099′	526	*A. squamata;* Ophiuroidea und.	Flank; hemipelagic sediments
TTR	TTR12	AT416	Gr	17.07.2002	35º21.870′	06º52.000′	695	*A. squamata*	Flank; coral framework
Meknès	TTR14	AT541	Gr	05.08.2004	34º59.103′	07º04.435′	703	*Amphiura* sensu Stöhr & Segonzac; *A. squamata*	Crater; mud breccia with strong smell of H_2S
	TTR15	AT581	Gr	28.07.2005	34º59.178′	07º04.353′	700	*A. squamata*	Crater; mud breccia
		AT585	K	28.07.2005	34º59.137′	07º04.343′	701	*A. squamata*	Crater; mud breccia
		AT586	Gr	28.07.2005	34º59.146′	07º04.380′	701	*A. squamata*	Crater; mud breccia
	MSM01-03	319	UB	14.05.2006	34º59.100′	07º04.439′	695	Amphiuridae und.	Crater; mud breccia
		321	UB	14.05.2006	34º58.796′	07º04.394′	732	*A. squamata*	Carbonate mound; shell ash and coral debris
		335	UB	15.05.2006	34º59.035′	07º04.552′	703	*A. squamata;* Ophiuroidea und.	Flank; coral debris, mud breccia
Yuma	TTR14	AT524	Gr	02.08.2004	35º24.973′	07º05.461′	960	*Amphiura hexabrachiatus*	Crater; mud breccia
	TTR16	AT604	Gr	29.05.2006	35º25.820′	07º06.330′	1030	*Amphiura* und.; *Amphiura* sensu Stöhr & Segonzac; *A. squamata;* Ophiuroidea juv.	Flank; mud breccia
		AT605	Gr	29.05.2006	35º25.046′	07º05.450′	975	Amphiuridae und.; *A. ingolfiana*	Crater; mud breccia
Ginsburg	TTR16	AT607	Gr	29.05.2006	35º22.677′	07º04.979′	983	*A. squamata*	Shell ash, carbonate slabs, crusts and coral debris
Jesuz Baraza	TTR12	AT391	Gr	09.07.2002	35º35.439′	07º12.264′	1105	*A. squamata*	
Darwin	TTR16	AT608	Gr	30.05.2006	35º23.531′	07º11.475′	1115	Amphiuridae und. *Amphiura* sensu Stöhr & Segonzac; *Amphiura hexabrachiatus*; *A. squamata*	Crater; shell ash; slabs and crusts.
Chechaouen	TTR16	AT610	Gr	30.05.2006	35º28.468′	07º15.477′	1177	Ophiuroidea juv.	Crater; mud breccia, crusts)
Cap. Arutyunov	TTR12	AT399	Gr	13.07.2002	35º39.805′	07º19.997′	1339	*A. squamata*	Crater rim; mud breccia
	TTR14	AT546		06.08.2004	35º39.692′	07º20.046′	1345	*Amphiura* sensu Stöhr & Segonzac	Crater; mud breccia with gas hydrates
	MSM01-03	182	BL	27.04.2006	35º39.642′	07º20.046′	1323	*A. squamata;* Ophiuroidea und.	Crater; mud breccia
		194	Gr	28.04.2006	35º39.282′	07º20.012′	1379	*A. squamata*	Flank; hemipelagic sediments
		195	Gr	28.04.2006	35º39.274′	07º20.013′	1390	*Ophiopristis cadiza*; *O. abyssicola; O. aristata; Amphiura hexabrachiatus*	Flank; coral thicket
		225	BL	04.05.2006	35º39.700′	07º20.010′	1320	*A. squamata*	Crater centre; mud breccia
		274	BL	10.05.2006	35º39.706′	07º20.001′	1321	*A. squamata*	Crater centre; mud breccia

Structure	Cruise	Station	Gear	Date (dd.mm.yy)	Latitude (N)	Longitude (W)	Depth (m)	Taxa	Observations
		344	FL	16.05.2006	35º39.697′	07º20.038′	1320	*A. squamata*	Crater; mud breccia
Carlos Ribeiro	MSM01-03	157	UB	23.04.2006	35º47.270′	08º25.360′	2200	Ophiuroidea juv.	Crater; mud breccia
Porto	MSM01-03	151	FL	25.04.2006	35º33.771′	09º30.516′	3862	*Ophiomusium lymani*	Crater; mud breccia
Carbonates and Corals									
W of Gibraltar	TTR14	AT550	D	07.08.2004	35º42.257′	06º30.000′	392	*O. balli; A. squamata*	Carbonate chimneys
		AT551	D	07.08.2004	35º42.769′	06º30.305′	393	*Amphiura* sensu Stöhr & Segonzac	Carbonate chimneys
		AT552	Gr	07.08.2004	35º42.816′	06º30.234′	428	Amphiuridae und.; *A. filiformis; A. squamata*	Carbonate chimneys in brawn marl
Vernadsky Ridge	TTR15	AT574	D	26.07.2005	35º02.982′	06º46.661′	508	*A. grandisquama*	Carbonate crusts and chimneys
Formosa Ridge	TTR12	AT388	Gr	08.07.2002	36º10.263′	07º43.819′	1079	*A. squamata*	Carbonate chimneys
		AT389	D	08.07.2002	36º10.123′	07º44.121′	1068	*A. squamata*	Carbonate chimneys
Pen Duick	TTR12	AT406	Gr	15.07.2002	35º18.148′	06º47.666′	550	*A. squamata; A. ingolfiana*	Coral framework
		AT407	Gr	15.07.1002	35º17.695′	06º47.082′	560	*A. filiformis*	Coral framework
	TTR14	AT565	Gr	09.08.2004	35º18.180′	06º47.656′	544	*A. squamata*	Coral framework, clay
	TTR16	AT600	Gr	28.05.2006	35º18.779′	06º48.453′	610	*A. grandisquama; Amphiura* sensu Stöhr & Segonzac; *Amphiura hexabrachiatus*; *A. squamata*	Mud breccia covered by coral debris and carbonate crusts
		AT602	Gr	28.05.2006	35º17.693′	06º47.089′	556	*A. filiformis; A. squamata; A. ingolfiana*	Hemipelagic sediments, shell debris cemented by carbonate crusts

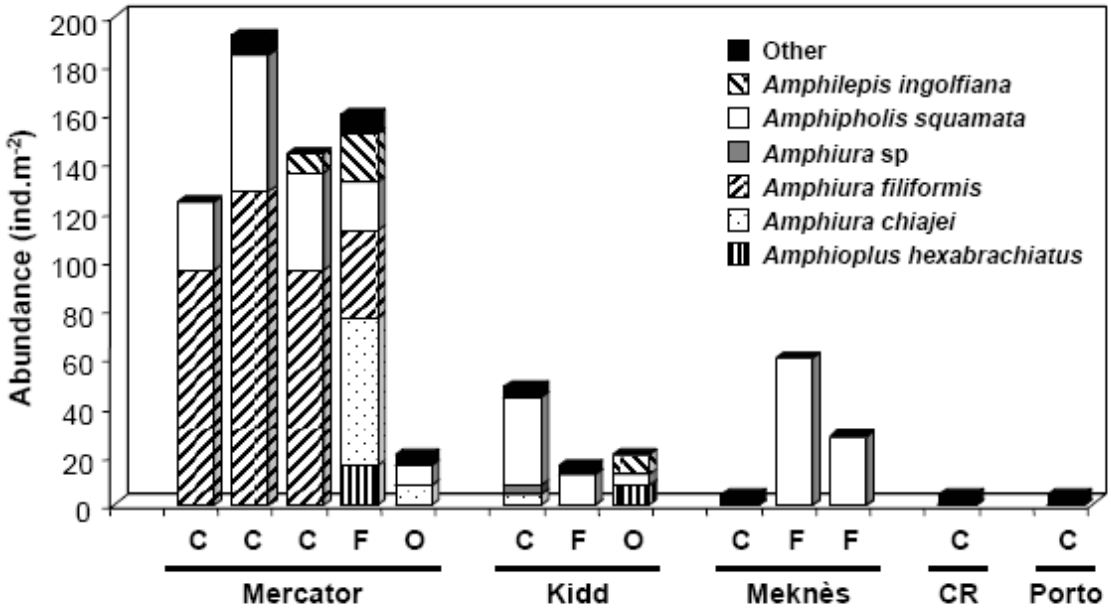

Figure 2.5.6. Abundance of Amphiuridae and other species estimated from box cores (A: 0.25m^{-2}) collected in Gulf of Cadiz mud volcanoes (shallowest at left to deepest at right). C: crater samples; F: flank samples; O: samples collected off the MV; CR: Carlos Ribeiro MV.

Previous records on deep-sea ophiuroids from reducing environments compiled from the available literature (Table 2.5.2) referred to four families, from which three, Ophiacanthidae, Ophiuridae and Amphiuridae are represented in cold seeps. These three families are also found in the Gulf of Cadiz but we can now add the occurrence of Ophiactidae formerly known to occur only in hydrothermal vents.

Only two species of the family Ophiacanthidae were found in the Gulf of Cadiz: the new species *Ophiopristus cadiza* and *Ophiacantha aristata* both from a single record in dead scleractinean corals, at the flank of a mud volcano. Representatives of *Ophiancantha*, *O. longispina* Stöhr & Segonzac 2005, only known from the type locality in a Barbados cold seep, do not show a special association to reduced environments (Stöhr & Segonzac 2005). *Ophienigma spinilimbatum*, found in association with tubeworms and mussels in four locations from Barbados and Gulf of Mexico (1947 to 3300m) is the only probable endemic to cold seeps (Stöhr & Segonzac 2005), *Ophiotetra valenciennesi rufescens* Koehler, 1896 was collected from scleractinean corals in the vicinity of a cold seep, and this is the only species known to occur in

vent, non-vent and cold seep sites (Stöhr & Segonzac 2005). Three other bathyal species of this family are known to occur in reduced environments: *Ophiomitra spinea* Verril 1850, collected from vent and non vent sites in the MAR, *Ophiolamina eprae*, endemic to vents in the East Pacific and *Ophioplinthaca chelys* (Wyville Thomson 1877) from the Barbados cold seeps (Stöhr & Segonzac 2005, 2006).

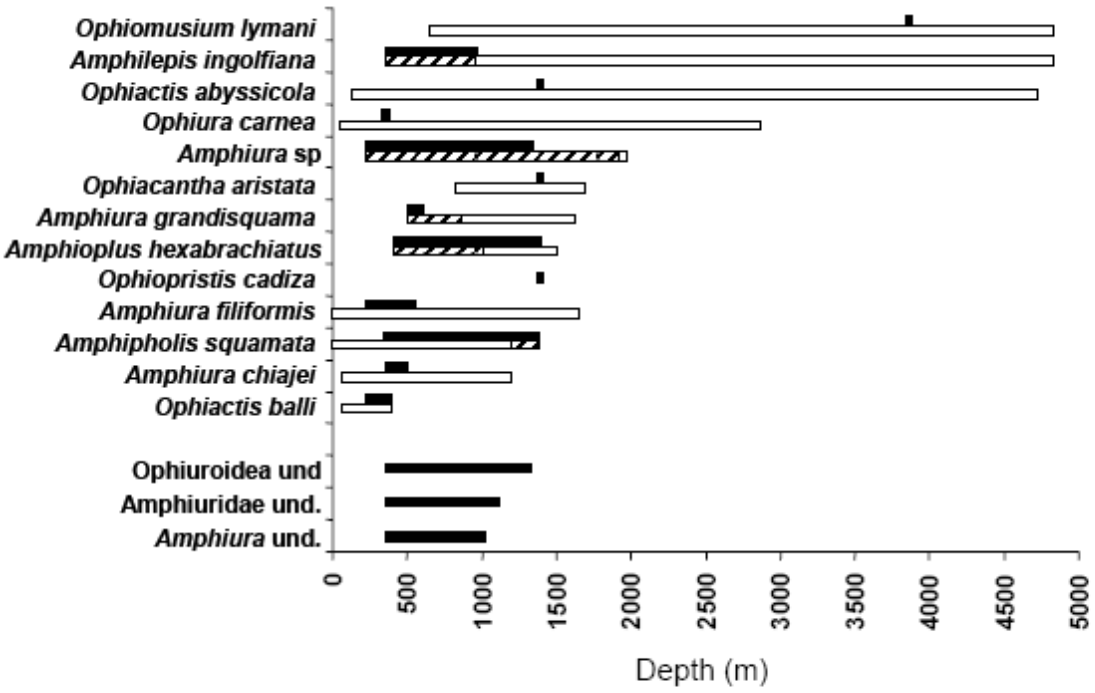

Figure 2.5.7. Bathymetric ranges of the ophiuroid species collected in the Gulf of Cadiz. Undetermined specimens (Ophiuroidea und., Amphiuridae und. and *Amphiura* und.) were mostly juveniles or damaged specimens. Black bars: depth ranges in the Gulf of Cadiz from the present study; White bars: previously known depth range; Striped bars: extended depth range by the records of the present study

The family Ophiactidae is represented in the Gulf of Cadiz by only a few records of two *Ophiactis* species, *O. abyssicola* found in dead scleractinean corals and *O. balli* associated to carbonates and sandstones. *Ophiactis abyssicola* and *Ophiactis tyleri* Stöhr and Segonzac, 2005 are reported from non vent MAR sites but the latter also occurs at vents (Stöhr & Segonzac 2005).

Amphiuridae is the best represented family in the Gulf of Cadiz with seven species (Figure 2.5.4), from which four belong to the genus *Amphiura*.

Amphiura sp. sensu Stöhr and Segonzac occurs mostly in the craters of mud volcanoes but it is also found associated to carbonate chimneys and crusts in areas with no evidence of present seepage activity. *Amphiura filiformis* has a wide distribution in the Gulf of Cadiz and was also found in mud volcanoes and carbonate areas but never in co-occurrence with *Amphiura* sp sensu Stöhr and Segonzac. *Amphiura chiajei* and *A. grandisquama* are less common but also show segregated distributions with the first associated only with soft sediments and the latter with hard substrates (carbonates and dead scleractinean corals) always off the crater. *Amphipholis squamata* is the most abundant and widespread ophiuroid in the Gulf of Cadiz, occurring in 70% of the samples and in all types of substrata. The remaining Amphiuridae species, *Amphilepis ingolfiana* and *Amphioplus hexabrachiatus* are also found in all types of substrates. There is no information available on Amphiuridae from vent habitats and the records from other cold seeps are restricted to the findings of undetermined *Amphioplus* sp. from the Gulf of Mexico (Stöhr & Segonzac 2005) and *Amphiura* sp. from Barbados (Stöhr & Segonzac 2005) and Alaska (Levin & Mendoza 2007). The distribution of *Amphiura* sp. sensu Stöhr and Segonzac, known to occur both in the Barbados (Stöhr & Segonzac 2005) and in the Gulf of Cadiz (this study) raises once more the question of population connectivity in cold seep species. Trans-Atlantic larval dispersal along the equatorial belt region was suggested to explain the occurrence of genetically related *Bathymodiolus* populations at West Africa and the Barbados cold seeps (Cordes *et al*. 2007; Olu-Le Roy *et al*. 2007; Génio *et al.* 2008). In the case of ophiuroids only known from reduced environments but with wide Atlantic distributions such as *Ophioctenella acies* (Stöhr & Segonzac 2005) and now *Amphiura* sp. sensu Stöhr and Segonzac, the lack of molecular studies do not exclude the possibility of cryptic species.

The family Ophiuridae was poorly represented in the samples taken from Gulf of Cadiz. Although Ophiuridae appear to be the dominant background megafauna recorded during video observations at the deeper mud volcanoes, only two specimens, one from *Ophiura carnea* and the other from *Ophiomusium lymani* were collected. Sumida *et al*. (2004) reported abundant specimens of *Ophiomusium anaelisae* Tommasi & Abreu, 1974, accounting for

72% of the fauna collected by dredging coral rubble and sediments from pockmarks in Santos Basin off SE Brazil, but the authors did not provide strong evidence of present active seepage. Two other Ophiuridae species, *Ophiocten gracilis* (G.O. Sars, 1871) and *Ophiopleura borealis* Danielssen & Koren, 1877, also dominate the background community at the Haakon Mosby MV (Gebruk *et al*. 2003). The same applies, for instance, to Carlos Ribeiro MV where ophiurids were abundant at the flanks and base of the mud volcano although they appeared more scattered in the crater (M.R. Cunha, pers. observation). Specimens in photographs taken with the ROV ISIS (NOCS) from this mud volcano were identified as large individuals of *Ophiomusium lymani*. Stöhr and Segonzac (2005, 2006) report one Ophiuridae species endemic to East pacific vents, *Spinophiura jolliveti*, and three from MAR: *Ophiura clemens* (Koehler 1904) and *Ophiocten centobi* Paterson, Tyler and Gage, 1982, that were found in vent and non- vent sites, and Ophioctenella acies. The distribution of *O. acies* ranges 1626 to 3500m depths and encompasses several vents from the MAR and cold seeps in the Western Atlantic; this species is considered by Stöhr and Segonzac (2005) as restricted to reduced environments.

2.5.5.Final remarks / Conclusions

The Gulf of Cadiz differs from other cold seep regions not only by the high number of species (up to five species co-occurring in a sampled area of 0.25m^{-2}, and up to six species per site) but also because Amphiuridae were dominant both in number of species (seven species) and abundance (up to 188 $ind.m^{-2}$ in a box core from Mercator crater). Furthermore bathymetric ranges of five Amphiuridae species (*Amphioplus hexabrachiatus*, *Amphilepis ingolfiana*, *Amphiura* sp. sensu Stöhr and Segonzac, *Amphiura grandisquama* and *Amphipholis squamata*) are extended, as well as the biogeographic ranges for two of these species: a new southern limit for the distribution of *Amphioplus hexabrachiatus* is set and the first site in the Eastern Atlantic for *Amphiura* sp. sensu Stöhr and Segonzac is recorded.

Table 2.5.2. Compilation of ophiuroid records from reduced environments. CS: cold seeps; V: hydrothermal vents; O: non-reducing environments. +: presence; (+): presence only in probably inactive adjacent habitats (eg. cold water corals and carbonates in the vicinity of seeps)

Species	O		V		CS	
Ophiacanthidae						
Ophiacantha aristata Koehler 1896	+	a)			(+)	b)
Ophiacantha longispina Stöhr & Segonzac, 2005					(+)	c)
Ophienigma spinilimbatum Stöhr & Segonzac, 2005					+	c)
Ophiolamina eprae Stohr & Segonzac, 2006			+	d)		
Ophiomitra spinea Verrill, 1885	+	c)	+	c)		
Ophioplinthaca chelys (WyvilleThomson, 1877)	+	a)			+	c)
Ophiopristis cadiza sp. nov.					(+)	b)
Ophiotreta valenciennesi Koehler, 1896	+	c)	+	c)	(+)	c)
Ophiactidae						
Ophiactis abyssicola (M. Sars,1861)	+	a)			(+)	b)
Ophiactis balli (Thompson, 1840)	+	a)			+	b)
Ophiactis tyleri Stöhr & Segonzac, 2005	+	c)	+	c)		
Amphiuridae						
Amphilepis ingolfiana Mortensen, 1933	+	a)			+	b)
Amphioplus sp					+	c)
Amphioplus hexabrachiatus Stöhr, 2003	+	e)			+	b)
Amphipholis squamata (Delle Chiaje, 1829)	+	f)			+	b)
Amphiura sp.					+	g)
Amphiura sp. sensu Stohr & Segonzac 2005					+	b) c)
Amphiura chiajei Forbes, 1843	+	a)			+	b)
Amphiura filiformis (O.F. Müller, 1776)	+	a)			+	b)
Amphiura grandisquama Lyman, 1869	+	a)			(+)	b)
Ophiuridae						
Ophiura carnea Lütken, 1858	+	a)			+	b)
Ophiura clemens (Koehler, 1904)	+		+	c)		
Ophioctenella acies Tyler *et al*., 1995			+	c)	+	c)
Ophiocten centobi Paterson, Tyler & Gage, 1982	+	c)	+	c)		
Ophiocten gracilis (G.O. Sars, 1871)	+				+	h)
Ophiomusium anaelisae Tommasi & Abreu, 1974					(+)	i)
Ophiomusium lymani W. Thomson 1873	+	a) f)			+	b)
Ophiopleura borealis Danielssen & Koren, 1877	+				+	h)
Spinophiura jolliveti Stohr & Segonzac 2006			+	d)		

a) Paterson 1985; b) this study; c) Stöhr & Segonzac 2005; d) Stohr & Segonzac 2006; e) Stöhr 2003; f) Alva & Vadon 1989; g) Levin & Mendoza 2007; h) Gebruk *et al*. 2003; i) Sumida *et al*. 2004.

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SECTION 3. TROPHIC RELATIONSHIPS AND CHARACTERIZATION OF MICROBIAL SYMBIOSES

Chapter 3.1.Nutritional relations in deep-sea macrofaunal communities from the mud volcanoes of the Gulf of Cadiz (NE Atlantic): a stable isotope approach

Abstract

The Gulf of Cadiz supports a diverse range of macrofaunal assemblages associated with hydrocarbon seep. The carbon, sulphur and nitrogen stable isotope signatures of dominant chemotrsymbiotic species and selected heterotrophic macrofauna from several mud volcanoes and adjacent habitats are described. Different species of Frenulata (Siboglinidae) and Bivalvia (Solemyidae, Lucinidae and Thyasiridae) showed a range of isotopic values consistent with either thiotrophic or methanotrophic nutrition. The analyses of heterotrophic fauna indicate that the species inhabiting the crater of the mud volcanoes derive the bulk of their nutrition from chemoautolithotrophic sources. However, the heterotrophic species living in adjacent habitats (carbonate concretions and cold-water scleractinean corals) showed isotopic signatures consistent with a diet based on phytoplankton-derived organic matter therefore suggesting that the export of chemotrophic production from mud volcanoes to these adjacent habitats is of little relevance.

Keywords: Stable isotopes, Chemosymbiotic species, Mud volcanoes, Gulf of Cadiz

Rodrigues CF & Cunha MR (in preparation) Nutritional relations in deep-sea macrofaunal communities from the mud volcanoes of the Gulf of Cadiz (NE Atlantic): a stable isotope approach.

Nutritional relations in deep-sea macrofaunal communities from the mud volcanoes of the Gulf of Cadiz (NE Atlantic): a stable isotope approach

3.1.1.Introduction

Inputs of material with nutritional value to the deep ocean include falls from large animals such as sharks and marine mammals and export of coastal land-derived material (debris of plants and macroalgae), However the main input of primary production into this environment is considered to be what is loosely referred as "marine snow": the downward flux from the epilagic zone of photosynthetically fixed material, aggregated detrital material (e.g. faecal pellets and plankton exoskeletons) and zooplankton (reviewed by Polunin *et al.* 2001). The supply of food is generally considered as the main limiting factor for most deep-sea communities (Gage & Tyler 1991) and consequently marine snow constitutes a valuable food resource for deep-sea microbes, metazoans and bathyal detritivores.

Besides photosynthetically-derived carbon, additional energy sources to the infauna that inhabit bathyal reduced environments include carbon fixed chemosynthetically in the seabed and made available through the activities of free-living or symbiotic bacteria (Levin & Michener 2002). Sulphur oxidation (thiotrophy) and methane oxidation (methanotrophy) are the primary chemosynthetic pathways that support carbon fixation of significance to animals (Conway *et al*. 1994; Van Dover 2000). Thiotrophic bacteria oxidize hydrogen sulphide and utilize the energy released to fix carbon dioxide into organic compounds. Similarly, methanotrophs use a reduced compound as an energy source, in this case methane, which is also the carbon source (Fisher *et al*. 1987). Nutrition of seep fauna varies, with thiotrophic and methanotrophic symbiotic bacteria fueling most of the megafaunal forms but macrofauna and most meiofauna are mainly heterotrophic. Macrofaunal food souces are largely photosynthetic-based at shallower seeps but reflect carbon fixation by chemosynthesis and considerable incoroporation of methane-derived carbon at deeper sites (Levin *et al*. 2000).

The different carbon fixation pathways described above involve distinct isotopic fractionation which makes stable isotope approaches particularly useful for elucidating nutritional status of organisms in vent and seep environments (Conway *et al.* 1994; Van Dover & Fry 1994). It was the fact that vent tube worms had very distinct $\delta^{13}C$ values relative to typical deep-ocean tissue carbon values that led Rau & Hedges (1979) to postulate that the dominant invertebrates had some non-photosynthetic food source. Since then carbon isotopic values have been used to help differentiate animals with chemoautotrophic symbionts (–20 to –40‰) from those with methanotrophic symbionts (≤–40‰) (Brooks *et al*. 1987; Kennicutt *et al*. 1992) and to identify the source methane pool as either thermogenic ($\delta^{13}C$ = –40 to –45‰) or biogenic ($\delta^{13}C \leq$ –45‰) methane (Sassen *et al*. 1999). Some $\delta^{15}N$ and $\delta^{34}S$ measurements have also been made on seep chemoautotrophs and heterotrophs (Brooks *et al.* 1987; MacAvoy *et al.* 2002). Chemoautotrophs tended to have lower $\delta^{15}N$ values (–5 to –12‰) than heterotrophs (2.8 to 13‰) or marine phytoplankton. The $\delta^{34}S$ values of the thiotrophic animals were depleted relative to the methanotrophic bivalves (13‰) and seep heterotrophs (13 to 16‰) (Brooks *et al.* 1987).

Three important aspects must be considered when comparing the isotopic compositions of different individuals or different species. First, if two organisms use the same dietary carbon and nitrogen sources they will have similar isotopic compositions. Second, organisms having the same isotopic compositions do not necessarily have the same diet, since they can have different diets with the same averaged isotopic compositions. Third, a difference in the isotopic compositions of two organisms reflects a difference in their diets. Stable isotopic compositions can therefore provide information on food resource use and partitioning, both interspecifically and intraspecifically (Levesque *et al*. 2003).

The main objectives of this study are: i) to determine the trophic status of selected macroinvertebrate species associated with mud volcanoes and adjacent habitats from Gulf of Cadiz by analyzing their $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ signatures; ii) to investigate the nutritional sources and pathways relevant for the trophic relationships in the benthic assemblage and iii) to determine the

carbon sources and the importance of chemoautolitotrophic primary production for the heterotrophic benthic macrofauna.

3.1.2.Material and methods

Study area. The Gulf of Cadiz is located in the NE Atlantic Ocean between 34ºN and 37º15'N and 6ºW to 9º45'W. It is enclosed by the southern Iberian and northern Moroccan margins, west of Gibraltar Strait. The geological history of the Gulf of Cadiz is intimately related to plate tectonic interaction between Southern Eurasia and North Africa and is driven by two major mechanisms: a) subduction associated with the westward emplacement of the Gibraltar Arc and formation of the Gulf of Cadiz accretionary wedge, probably not active at present and b) oblique lithosphere collision between Iberia and Nubia, active at present and causing active thrusting (Zitellini *et al*. 2009). It is now well established that the whole area is under compressive deformation and that mud volcanism and processes associated with the escape of hydrocarbon-rich fluids sustain a broad diversity of chemosynthetic assemblages. This extensive area (Figure 3.1.1) encompasses over forty mud volcanoes (confirmed by coring), at depths ranging from 200 to 4000m (Mazurenko *et al*. 2002; Pinheiro *et al*. 2003; Magalhães 2007), and active methane seepage has been documented on several locations (Kenyon *et al*. 2000; Gardner 2001; Stadnitskaia *et al*. 2006; Hensen *et al*. 2007; Niemann *et al*. 2007).

Sample collection and preparation: samples for stable isotope analysis were collected during numerous cruises (TTR12, TTR14, TTR15, TTR16, MSMerian01-03, Microsystems 2006, Microsystems 2007 and JC10) in several mud volcanoes and adjacent habitats (carbonate concretions and cold-water coral thickets) from the Gulf of Cadiz (Table 3.1.1). Whenever possible the specimens were dissected on board and the different tissues were frozen for further processing in the laboratory. The samples included decapod muscle, bivalve gill, mantle and foot tissues and entire specimens of smaller species.

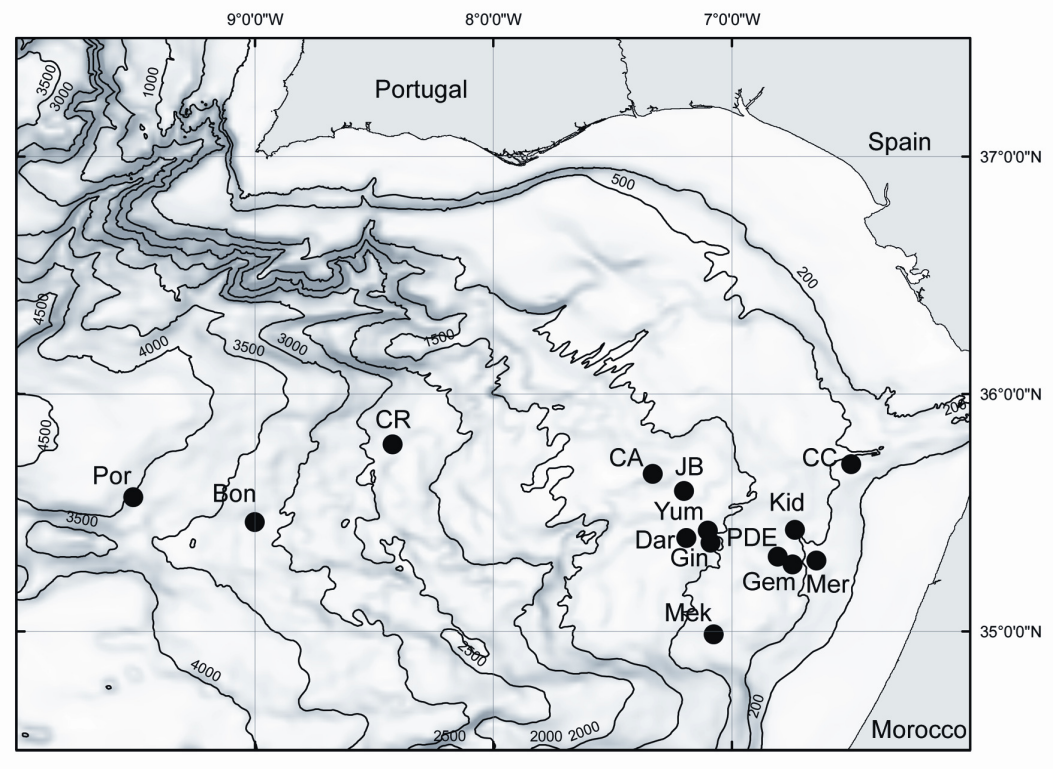

Figure 3.1.1. Map of the study area where specimens were collected for stable isotope analysis.

Stable isotopic analyses. Samples arriving at the laboratory were lyophilized and homogenized with a mortal-and-pestle grinding tool and aliquots were separated for $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ analyses. The ground sample for carbon analysis was acidified with HCl (1M) until no further bubbling occurred; the sample was re-suspended in distilled water, centrifuged and the supernatant was discarded, finally the sample was dried at 60ºC. The ground sample for sulphur analysis was re-suspended in distilled water, shaked for 5 minutes, centrifuged and the supernatant was discarded; this procedure was repeated 3 times and finaly the sample was dried at 60ºC and re-grinded.

The samples were analyzed at the ISO-Analytical laboratory (United Kingdom) using the method EA-IRMS (elemental analysis - isotope ratio mass spectrometry).

The isotope compositions are reported relative to standard material and follow the same procedure for all stable isotopic measurements (equation 3.1.1).

(Equation 3.1.1) $$\delta X = \left[\frac{R_{Sample} - R_{s\tan dard}}{R_{s\tan dard}} \right] \times 10^3 \left({}^{0}/_{00} \right)$$

where E is the element analyzed (C, N or S), x is the molecular weight of the heavier isotope, and y the lighter isotope (x=13, 15, 34 and y=12, 14 and 32 for C, N and S, respectively). The standard materials to which the samples are compared are the marine limestone fossil, Pee Dee Belemnite (PBD) for carbon (Craig 1953); atmospheric air for nitrogen (Mariotti 1983). and triolite from the Canyon Diabolo iron meteorite (CTD) for sulphur(Krouse 1980).

The average value and standard error is presented when different specimens were available at the same site. Alternatively different tissues were also used to calculate average values.

3.1.3.Results

During this study stable isotope values of several specimens of over 30 species from different sites and habitats were determined and presented in Figure 3.1.2 and Table 3.1.1.

Overall, $\delta^{13}C$ average values for specimens collected showed a clear segregation between mud volcanoes and adjacent habitats (cold water corals and carbonate concretions). In the fauna from mud volcanoes, $\delta^{13}C$ values were clearly more depleted (-52.38‰ to -27.97‰ in chemosymbiotic fauna; -37.62‰ to -19,61‰ for heterotrophic fauna) than in the fauna collected from the adjacent habitats (-22.14‰ to -5.74‰). The segregation was also clear for $\delta^{34}S$ values, ranging -27.61‰ to 17.71‰ and -7.79‰ to 19.88‰ rspectively in chemosymbiotic and heterotrophic fauna from mud volcanoes and less depleted values (13.76‰ to 24.31‰) in the specimens from adjacent habitats. For $\delta^{15}N$ a greater overlap was observed but consistently lower values were estimated for the species collected in the mud volcanoes (-3.38‰ to 10.46‰ in mud volcanoes; 6.91‰ to 15.31‰ in adjacent habitats)

The isotopic signatures of the chemosymbiotic species are shown in more detail in separate plots (detail Figure 3.1.2). Although analyses of different tissues were carried out in bivalve specimens no significant differences in

isotopic values were observed and therefore only the average results are presented.

The most depleted $\delta^{13}C$ values were measured in *Bathymodiolus mauritanicus* from Darwin MV (-52.38±0.98‰) and in *Siboglinum* specimens from Captain Arutyunov MV (-49.76‰). However the $\delta^{34}S$ signatures of the two species were quite different (16.77±0.20‰ and 17.71±0.02‰ for *B. mauritanicus*; -8.20‰ and 3.57‰ for *S. poseidoni*). All other chemosymbiotic species showed higher but consistently depleted $\delta^{13}C$ values between -20 and -40‰. The signature values varied mostly with the species and origin of the specimens being usually higher in specimens collected from deeper mud volcanoes. *Acharax* sp. nov showed values from -27.97‰ to -29.31‰ in the shallower mud volcanoes and -31.62‰ to -34.5‰ in the deeper ones. The values for *Thyasira vulcolutre*, only found in the deeper mud volcanoes were more depleted (-36.0‰ to -34.4‰) than the ones for *Petrasma* sp. nov (-33.99 to -32.15‰) and *Lucinoma* sp. nov. (-29.8‰) that occur only at shallower mud volcanoes. The frenulates showed often more depleted $\delta^{13}C$ signatures than the bivalves collected from the same mud volcanoes.

The $\delta^{34}S$ signatures of the chemosymbiotic species varied widely, especially in the bivalves (*Thyasira vulcolutre* -27.61‰ to 1.32‰; *Acharax* sp. nov.: -25.66‰ to 11.89‰; *Petrasma* sp.nov: -10.75‰ to 2.23‰; Frenulata: -16.85‰ to 6.55‰). Except for *B. mauritanicus* only a small specimen of *Acharax* sp. nov. from Porto MV and a few siiboglinid specimens from Gemini MV and Pen Duick Escarpment showed $\delta^{34}S$ values higher than 5‰

Except for one specimen of *Petrasma* sp.nov. from Gemini MV and two specimens of *Siboglinum* sp. collected in Gemini and Pen Duick Escarpment chemosymbiotic species showed depleted $\delta^{15}N$ signatures (<5‰)

Among the species of the remaining fauna several polychaete taxa were also analysed, some collected in the mud volcanoes (especially from Captain Arutyunov where they were particulary abundant) and some from the adjacent habitats (mostly from the Pen Duick Escarpment). The segregation of samples when $\delta^{13}C$ and $\delta^{34}S$ values are plotted together (Figure 3.1.3) is very clear

showing the group from the mud volcanoes with depleted isotopic signatures and the other group in the opposite quadrant of the plot.

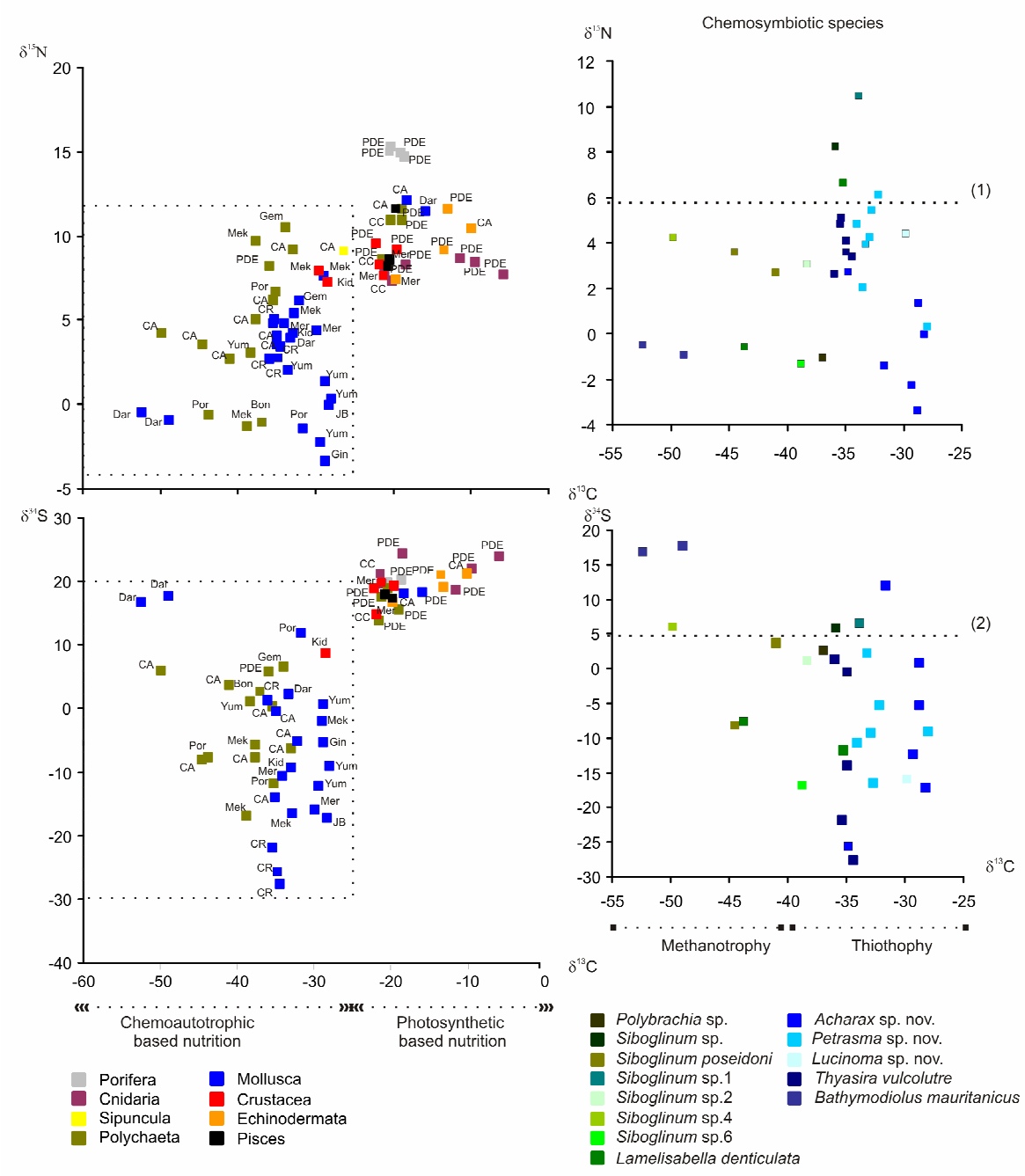

Figure 3.1.2. Isotope plots of $\delta^{13}C$ vs $\delta^{15}N$ and $\delta^{13}C$ vs $\delta^{34}S$ values for the examined taxa from different sites. CA: Captain Arutyunov; CR: Carlos Ribeiro; Dar: Darwin; Gem: Gemini; Gin: Ginsburg; JB: Jesuz Baraza; Kid: Kidd; Mek: Meknès; Mer: Mercator; Por: Porto; Yum: Yuma; PDE: Pen Duick Escarpment; CC: West of Gibraltar (1) $\delta^{15}N$>6 ‰ – Chemosynthetic symbionts are not probably present (Levin & Michener 2002); (2) $\delta^{34}S$<5 ‰ can be used to infer a thiotrophic mode of nutrition (Vetter & Fry 1998).

Within the specimens collected from the mud volcanoes the glycerids showed the highest $\delta^{15}N$ value (9.67‰) and the ampharetids the lowest (5.03‰). In the adjacent habitats $\delta^{15}N$ values for polychaetes varied between 8.55‰ and 11.69‰.

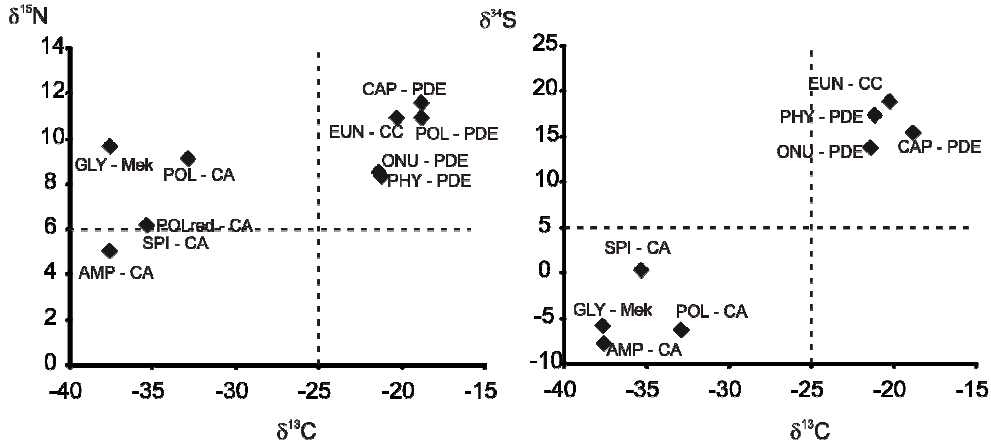

Figure 3.1.3. Stable isotope values for Polychaeta from Gulf of Cadiz. Families: AMP, Ampharetidae; CAP, Capitellidae; EUN, Eunicidae; GLY, Glyceridae; ONU, Onuphidae; POL: Polychaeta Undetermined; POLred: Polychaeta undetermined red; SPI, Spionidae. Mud volcanoes and adjacent habitats: CA, Captain Arutyunov MV; CC, Chimneys from Gibratar Channel; Mek, Meknès MV; PDE, Pen Duick Escarpment. δ15N>6 ‰ – Chemosynthetic symbionts are not probably present (Levin & Michener 2002); δ34S<5 ‰ can be used to infer a thiotrophic mode of nutrition (Vetter & Fry 1998)

Decapods from two species were also analysed: *Monodaeus couchi* was collected from Kidd MV and from a carbonate chimney field West of Gibraltar and *Cymonomus granulatus* was collected from Meknès MV. All specimens showed consistently high $\delta^{15}N$ values (7.21‰ to 8.25‰) but the specimens of both species collected from the mud volcanoes had significantly depleted $\delta^{13}C$ signatures (-28.34‰ and -29.52‰) in relation to the specimen collected from the carbonates (-21.76‰). The $\delta^{34}S$ values measured in *M. couchi* showed the same trend: 14.65‰ in carbonates and lower values in the mud volcano (8.73‰). The gastropod *Neptunea contraria* also collected from the crater of Meknès MV showed an isotopic signature very close to the decapods ($\delta^{15}N$: 7.60‰; $\delta^{13}C$: 28.26‰) with only more depleted $\delta^{34}S$ values (-2.11‰).

Table 3.1.1. Stable isotope values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ (average ± standard error) for the different faunal species collected in different mud volcanoes and adjacent habitats in the Gulf of Cadiz. Mud volcanoes: Bon. Bonjardim; CA. Captain Arutyunov; CR. Carlos Ribeiro; Dar: Darwin; Gem. Gemini; Gin. Ginsburg; JB: Jesuz Baraza; Kid. Kidd; Mek. Meknès; Mer. Mercator; Por. Porto; Yum. Yuma; PDE: pen Duick Escarpment; VR. Vernadsky Ridge location and values determined.

Species	$\delta^{13}C$ Ave.	±SE	$\delta^{15}N$ Ave.	±SE	$\delta^{34}S$ Ave.	±SE	Site	Cruise & Station	Latitude (N)	Longitude (W)	Depth (m)
Porifera											
Porifera und.	-20.46		14.97				PDE	M2006-19	35°11.310'	7°4.300'	908
Porifera und.	-18.91		14.88				PDE	M2006-40A	35°18.910'	6°47.030'	560
Porifera und.	-20.31		15.31		19.82		PDE	M2006-35	35°18.920'	6°47.210'	542
Porifera und.	-18.55		14.67		20.27		PDE	M2006-40A	35°18.910'	6°47.030'	560
Cnidaria											
Actiniaria und.	-21.26		6.91		18.45		PDE	M2006-02	35º17.770'	6º47.210'	420
Isidella elongata	-20.10		8.39		21.12		VR	TTR14-AT574D	35º25.982'	6º46.661'	508
	-5.74		7.31				PDE	M2006-34	35°18.920'	6°47.410'	542
	-9.41		7.65		23.87		PDE	M2006-44	35°18.900'	6°48.900'	640
Octocorallia und.	-18.41		8.45		21.96		PDE	M2006-40A	35°18.910'	6°47.030'	560
Octocorallia und.	-11.39		8.30		24.31		PDE	M2006-54	35°18.090'	6°48.120'	634
Hydrozoa und.	-21.10	±0.03	8.24	±0.39	19.14	±0,51	PDE	M2006-02	35º17.770'	6º47.210'	420
Mollusca											
Bentharca asperula	-18.23		12.10		18.09		CA	MSM01.03-195	35º39.274'	7º20.013'	1390
Bathyarca phyllipiana	-15.87		11.45		18.34		Dar	JC10-Dive 33	35º23.523’	7º11.513’	1109
Acharax sp. nov	-28.19	±0.38	-0.05	±0.06	-17.28	±0,16	JB	TTR12-AT391GR	35º35.439'	7º12.264'	1105
	-27.97	±0.32	0.29	±0.28	-9.20	±1,16	Yum	TTR14-AT524Gr	35º24.973'	7º05.461'	960
	-31.62	±0.27	-1.44	±0.16	11.89	±1,92	Por	MSM01.03-145	35º33.699'	9º30.437'	3860
	-34.75	±0.28	2.73	±0.20	-25.66	±1,71	CR	JC10-Dive 57	35º47.250’	8º25.324’	2175
	-28.77	±0.61	-3.38	±0.26	-5.40	±1,02	Gin	TTR16-AT607Gr	35º22.677'	7º04.979'	983
	-29.31	±0.62	-2.29	±0.32	-12.33	±1,38	Yum	TTR16-AT604Gr	35º25.820'	7º06.330'	1030
	-28.73	±0.27	1.33	±0.22	0.76	±0,71	Yum	TTR16-AT605Gr	35º25.046'	7º05.450'	975
Petrasma sp. nov.	-32.91	±0.76	4.20	±0.33	-9.39	±1,27	Kid	TTR14-AT528Gr	35º25.304'	6º43.972'	489
	-33.99	±0.35	4.82	±0.27	-10.75	±1,95	Mer	TTR15-AT569Gr	35º17.917'	6º38.717'	358
	-32.71	±0.45	5.43	±0.17	-6.50	±3,11	Mek	TTR15-AT586Gr	34º59.146'	7º04.380'	701
	-33.53	±1.14	2.01	±0.41			Yum	TTR16-AT604Gr	35º25.820'	7º06.330'	1030

Species	$\delta^{13}C$ Ave.	±SE	$\delta^{15}N$ Ave.	±SE	$\delta^{34}S$ Ave.	±SE	Site	Cruise & Station	Latitude (N)	Longitude (W)	Depth (m)
	-32.15	±0.25	6.10	±0.52	-5.28	±0,84	Gem	M2007_19	35º16.916'	6º45.469'	430
	-33.24	±0.15	3.91	±0.18	2.23	±1,01	Dar	JC10-Dive 33	35º23.523’	7º11.513’	1109
Bathymodiolus mauritanicus	-52.38	±0.98	-0.52	±0.11	16.77	±0,20	Dar	TTR16-AT 608Gr	35º23.531'	7º11.475'	1115
	-48.90	±1.36	-0.96	±0.28	17.71	±0,02	Dar	JC10-Dive 33	35º23.523’	7º11.513’	1109
Lucinoma sp. nov.	-29.80	±0.36	4.38	±0.30	-15.96	±2,55	Mer	TTR15-AT569Gr	35º17.917'	6º38.717'	358
Thyasira vulcolutre	-35.95	±0.80	2.64	±1.17	1.32	±0,43	CR	TTR16-AT615Gr	35º47.238'	8º25.272'	2200
	-35.31	±0.40	5.05	±0.78	-21.92		CR	MSM01.03-169.	35º47.256'	8º25.361'	2199
	-34.39	±0.33	3.41	±0.71	-27.61	±1,74	CR	MSM01.03-157	35º47.270'	8º25.360'	2200
	-35.39	±1.00	4.82	±0.88			CR	JC10-Dive 57	35º47.250’	8º25.324’	2175
	-34.90	±0.01	3.59	±1.28	-0.59	±0,62	CA	MSM01.03-212.	35º39.673'	7º19.974'	1317
	-34.94	±0.60	4.07	±0.90	-14.04	±2,38	CA	MSM01-03-217.	35º39.643'	7º20.046'	1321
Neptunea contraria	-28.86		7.60		-2.11		Mek	Geob-12748	35º58.85’	7º04.39’	722
Sipuncula	-26.31		9.09				CA	MSM01.03-181.	35º39.642'	7º20.046'	1323
Polychaeta											
Ampharetidae sp.nov.	-37.62		5.03		-7.79		CA	MSM01.03-217.	35º39.643'	7º20.046'	1321
Capitellidae	-18.80		11.60		15.44		PDE	M2007-07	35º14.618'	6º37.980'	432
Eunice vittata	-20.26		10.93		18.82		CC	TTR14-AT 574D	35º25.982'	6º46.661'	508
Glyceridae	-37.63		9.67		-5.85		Mek	TTR15-AT586Gr	34º59.146'	7º04.380'	701
Hyalonecia tubicola	-21.40		8.55		13.76		PDE	M2005-46	35°13.860'	6°36.600'	228
Phyllodocidae	-21.17		8.37		17.42		PDE	M2006-46B	35°13.850'	6°36.590'	227
Spionidae	-35.37		6.20		0.26		CA	MSM01.03-225	35º39.700'	7º20.010'	1320
Polychaeta und.	-18.85		10.94				PDE	M2006-42	35°18,920'	6°48,390'	637
Polychaeta und	-35.39		6.17				CA	MSM01.03-181	35º39.642'	7º20.046'	1323
Polychaeta und	-32.89		9.16		-6.30		CA	MSM01.03-344	35:39.697'	7:20.038'	1320
Polybrachia sp.	-36.91		-1.06		2.53		Bon	TTR15-AT597Gr	35º27.563'	9º00.030'	3061
Siboglinum poseidoni	-41.01		2.67		3.57		CA	TTR14-AT546Gr	35º39.692'	7º20.046'	1345
	-44.46		3.59		-8.20		CA	TTR14-AT544Gr	35º39.707'	7º20.012'	1330
Siboglinum sp.4	-49.76		4.20		5.97		CA	MSM01.03-190.1	35º39.665'	7º19.970'	1322
Siboglinum sp.1	-33.84	±0.71	10.46	±1.75	6.55		Gem	M2007_15	35º17.905'	6º44.286'	444
Siboglinum sp.6	-38.74		-1.31		-16.85		Mek	TTR15-AT586Gr	34º59.146'	7º04.380'	701
Lamelisabella denticulata	-43.67		-0.62		-7.70		Por	MSM01.03-145	35º33.699'	9º30.437'	3860
	-35.18		6.64		-11.82		Por	MSM01.03162	35º33.738'	9º30.492'	3863
Siboglinum sp.2	-38.25		3.05		1.10		Yum	TTR16-AT 605Gr	35º25.046'	7º05.450'	975
Siboglinum sp.	-35.87		8.22		5.73		PDE	M2006-38A	35°19.090'	6°46.400'	494

Species	$\delta^{13}C$ Ave.	±SE	$\delta^{15}N$ Ave.	±SE	$\delta^{34}S$ Ave.	±SE	Site	Cruise & Station	Latitude (N)	Longitude (W)	Depth (m)
Crustacea											
Euphausiacea	-21.19	±0.68	7.60	±0.28	19.88	±0,12	Mer	TTR15-AT569Gr	35º17.917'	6º38.717'	358
Amphipoda und.	-22.14		9.50		18.86		PDE	M2006-02	35º17.770'	6º47.210'	420
Cirolanidae	-19.50		9.14		19.21		PDE	M2007-08	35º14.111'	6º37.988'	428
Monodaeus couchi	-28.34	±2.87	7.21	±0.71	8.73		Kid	TTR14-AT528Gr	35º25.304'	6º43.972'	489
	-21.76		8.25		14.65		VR	TTR15-AT574D	35º25.982'	6º46.661'	508
Cymonomus granulatus	-29.52		7.92				Mek	TTR15-AT586Gr	34º59.146'	7º04.380'	701
Echinodermata											
Ophiopristis cadiza	-9.95		10.44		21.12		CA	MSM01.03-195	35º39.274'	7º20.013'	1390
Ophiuroidea	-19.61		7.42		16.65		Mer	TTR15-AT569Gr	35º17.917'	6º38.717'	358
Holothuridae	-13.00		11.59		19.05		PDE	M2007-53A	35º19.294'	6º47.281'	652
Crinoidea	-13.46		9.15		20.98		PDE	M2006-40	35°18.900'	6°47.020'	542
Pisces											
Undetermined	-19.70		11.59	±0.39	17.19		CA	MSM01.03-195	35º39.274'	7º20.013'	1390
Undetermined	-20.44		8.54				Mer	JC10-Dive28	35º17.914’	6º38.709’	354\
Undetermined	-20.59		8.20		17.89		PDE	M2007-07	35º14.618'	6º37.980'	432

One of the samples from Captain Arutyunov (MSM01.03-195) was collected from a coral thicket at the lower flank of the mud volcano. The ophiuroid *Ophiopristis cadiza*, the bivalve *Bentharca asperula* and a small fish of undetermined species were analysed. None of these species showed signs of significantly depleted $\delta^{13}C$ or $\delta^{34}S$ signatures. In the plots shown in Figure 3.1.2 these species plot together with all the other specimens collected from Pen Duick Escarpment and carbonate chimney fields.

3.1.4.Discussion

The collection of several specimens of different taxa for stable isotope analyzes in the Gulf of Cadiz allowed an overview of the trophic status of some of the most common fauna found in the mud volcanoes and adjacent habitats. The most obvious result of this study was the clear segregation of isotopic signatures of the fauna inhabiting mud volcanoes from the fauna living in the adjacent habitats pointing out localized importance of the chemotrophic production for the benthic food webs in the mud volcanoes with no relevant influence for the adjacent habitats.

The values determined for all the species collected form the adjacent habitats are consistent with a diet based on phytoplankton-derived organic matter, typically with $\delta^{13}C$ signatures of -15‰ to -25‰ (Fry & Sherr 1984). Organisms with a diet based on carbon produced by marine chemoautotrophs will be expected to have $\delta^{13}C$ values considerably more negative than organisms whose diet is based on photossynthetically fixed carbon (Brooks *et al.* 1987; Fisher 1990; Robinson & Cavanaugh 1995).

Values of $\delta^{34}S$ below 5‰ can be used to infer a thiotrophic mode of nutrition (Vetter & Fry, 1998) reflecting the isotopic signature of the sulphide source (Fisher 1995). Seep macrofauna can obtain chemosynthetically fixed organic matter by (1) translocation from sulphide- or methane-oxidizing symbionts, (2) feeding on free-living chemoautotrophic bacteria, or (3) consuming another animal that obtains nutrition through (1) or (2) (MacAvoy *et al.* 2002). Sulphide is clearly the major energy source for the invertebrate endosymbioses prevalent in the Gulf of Cadiz area but comparisons with other

seep areas are difficult because this isotope is rarely determined in the available studies.

The narrow ranges of the carbon signatures of the species collected in each mud volcano and the variability between the signatures of the fauna from different mud volcanoes in interpreted as corresponding to the spatial variability in the carbon source, its specific $\delta^{13}C$ signature and the different pathways utilized by the species.

Carbon isotopic values have been used to help differentiate animals with sulphur-oxidizing symbionts from those with methano-oxidizing symbionts; $\delta^{13}C$ values between lower than -20 ‰ are consistent with chemoautotrophic symbiont-bearing animals and values lower -40 ‰ are particularly indicative of the presence of methanotrophic symbionts (Brooks *et al*. 1987; Fisher 1995; Kennicutt *et al.* 1992). The bivalvia tissues showed no clear differences in their isotopic signatures in contradiction to the results obtained by Cary *et al*. (1989) that suggested a high degree of carbon flow from bacterial symbiont to host. The depleted values determined for the bivalves in the Gulf of Cadiz are in line with data for other bivalves with thiotrophic symbionts using CO_2 as their carbon source (Southward *et al*. 2001) and bivalves with methanotrophic symbionts (Fisher 1990).

Sulphur-based chemoautotrophic symbiotic bacteria have been documented from gill tissues of all species of solemyids investigated to date (Barry *et al*. 2000). Solemyids oxidize sulphide to a non-toxic oxidation product thiosulphate which is then transported to gill tissues for further oxidation to support bacterial chemosynthesis (Barry *et al*. 2000). *Acharax* specimens are known to rely only on chemoautotrophic nutrition. On the other hand, *Solemya velum* has a gut which is small but apparently functional (Yonge 1939). Differences on nitrogen values between Solemyid species suggest that they are using different chemical species of nitrogen, tapping different pools of nitrogen, or discriminating differently after acquisition of their nitrogen source. Furthermore, *Solemya* is known to obtain most of its organic carbon from symbiont carbon fixation in spite of the presence of a reduced gut and its diminished ability to filter feed (Scott & Cavanaugh 2007). Isotopic values of $\delta^{13}C$ of our *Petrasma* sp. nov. specimens are similar to the

ones found for *Solemya* sp. (Conway *et al*. 1989) but our results of $\delta^{15}N$ are much higher probably reflecting differences in the organic nitrogen sources. Levin & Michener (2002) measured values of $\delta^{13}C$ for *Acharax* specimens from the Gulf of Alaska (-35.47‰ and -33.46‰) that are close to the ones measured in the Gulf of Cadiz for the *Acharax* specimens of the deeper mud volcanos but slightly more depleted than the values from the shallower mud volcanoes.

The $\delta^{13}C$ values measured in lucinid specimens are also in the range of values reported elsewhere for lucinids harbouring thiotrophic symbionts (reviewed by Fisher 1990). For *Thyasira vulculotre* determination of the stable carbon isotope composition of the tissues indicates that the autotrophic bacteria also make a substantial contribution to the nutrition of the host. Thyasirids are known to have thiotrophic symbionts but also can alternate their way of feeding if the usual resource is not available. Chemoautotrophy is usually considered to be more important in the nutrition of thyasirids than of lucinids (Van Dover & Fry 1989).

The specimens of *Bathymodiolus mauritanicus* collected in the Gulf of Cadiz were consistent with methanotrophic nutrition. Deep-sea mussels have been reported to harbour thioautotrophs and/or methanotrophs as endosymbionts in their gills (Nelson & Fisher 1995) and several species of *Bathymodiolus* with dual symbiosis have been identified from the Atlantic Basins (Cavanaugh *et al*. 1992; Duperron *et al.* 2006; Fiala-Médioni *et al*. 1996). Stable isotope values of *B. mauritanicus* were extremely depleted in $\delta^{13}C$ and suggested methanotrophic nutrition but molecular studies also revealed the presence of sulphur-oxidizing bacteria. Therefore, a dual symbiosys as reported for *Bathymodioulus azoricus* (Duperron *et al.* 2006) is likely to occur in *B. mauritanicus*.

Average isotopic carbon signatures for Frenulata in Captain Arutyunov were compatible with methanotrophic nutrition, but average sulphur carbon values were also compatible with thiotrophic occurrence. Due to their morphological similarity different species may have been pooled together which dificults the interpretation of the results. Values of $\delta^{13}C$ up to -45.83‰ were also determined in the frenulate *Siboglinum atlanticum*, which does not contain

methane-oxidizing bacteria (Gebruk *et al*. 2003), however unpublished results (Chapter 3.3) suggest the occurrence of methane oxidizing bacteria in some of the specimens collected in Captain Arutyunov (*S. poseidoni*).

Carbon isotopic values have also been used to identify the source methane pool as either thermogenic ($\delta^{13}C$ = –40 to –45‰) or biogenic ($\delta^{13}C \leq$ -45‰) methane (Sassen *et al.* 1999). The $\delta^{13}C$ signature in the specimens of *Siboglinum poseidoni* (methanotrophic) collected from Captain Arutyunov are consistent with the inferred thermogenic origin of methane in the Gulf of Cadiz (Nuzzo *et al.* 2006) although slightly lighter signatures were found in the *Bathymodiolus mauritanicus* from Darwin (-52.38‰ and -48.9‰) .The values of $\delta^{13}C$ for other species in different mud volcanoes all support the hypothesis for a chemoautotrophic-sourced host carbon nutrition.

Values of $\delta^{15}N$ higher than 6‰ indicate that chemosynthetic symbionts are not probably present (Levin & Michener 2002). Low $\delta^{15}N$ values are possible due to assimilation of isotopically light nitrate or ammonium by the symbionts or, in the case of the very negative values, perhaps fixation of N_2 (Fisher 1995). The lower $\delta^{15}N$ values in specimens from the mud volcanoes indicate that organic nitrogen is of local origin. Autotrophic bacteria are presumably this source of nitrogen and particularly in symbiont-containing invertebrates the isotopic signature of bacteria-rich tissues may be indicative of the $\delta^{15}N$ compositions of their endosymbiont populations (Van Dover & Fry 1989). The more depleted signatures of $\delta^{15}N$ in the Gulf of Cadiz were determined for the chemosymbiotic bivalves and frenulates. The highest values determined in the mud volcanoes were for glycerid polychaetes, decapods and the gastropod *Neptunea contraria* indicating a high trophic level of these species. Intermediate values consistent with (bacterial) grazing or deposit feeding were determined for Ampharetidae and Spionidae polychaetes.

The interpretation of the results on the trophic status and possible nutritional relationships of the analysed species was the base for the empirical model of the benthic food web in the Gulf of Cadiz shown in Figure 3.1.4.

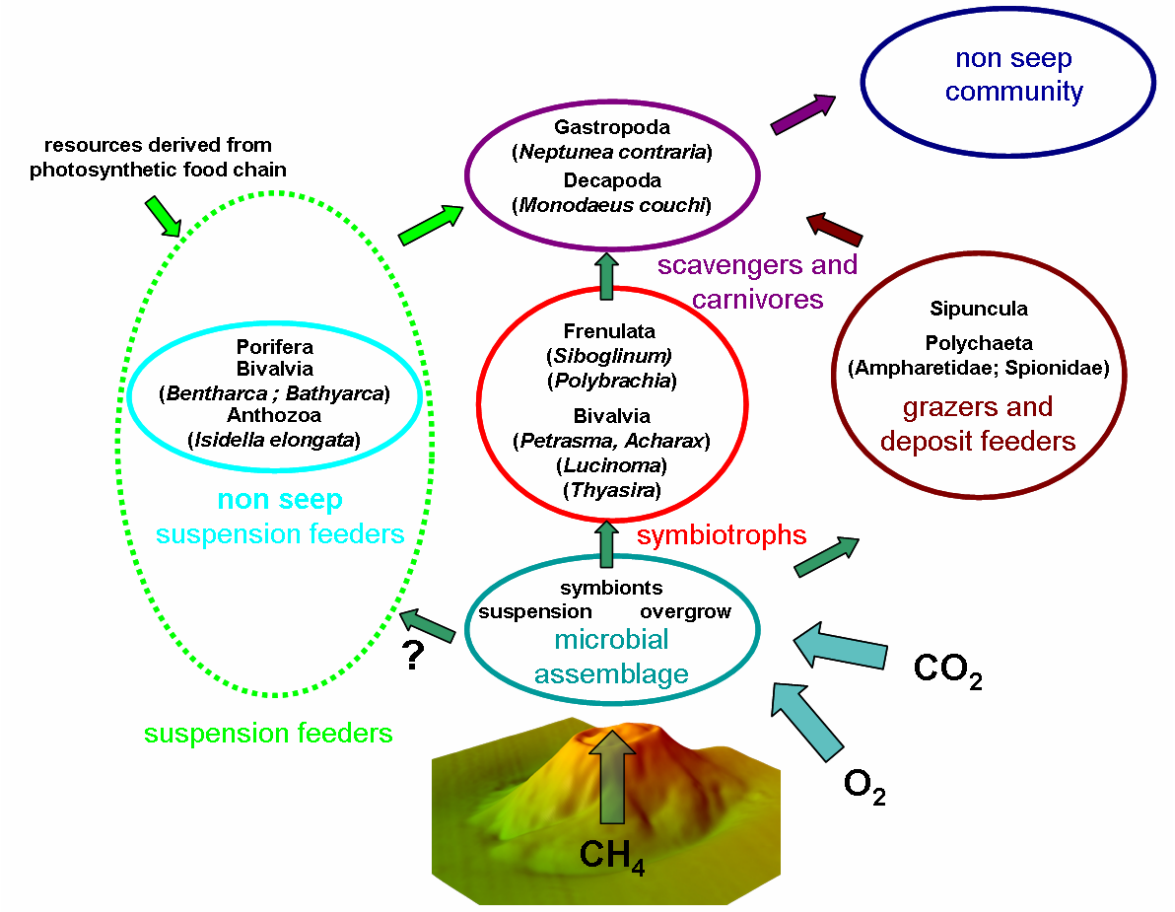

Figure 3.1.4. Simplified food web model for mud volcanoes from the Gulf of Cadiz based on the interpretation of the isotopic signatures of major players in the different trophic groups . The question mark indicates that a significant contribution of chemotrophic production to the nutrition of suspension feeders in adjacent habitats is unlikely.

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Chapter 3.2.Chemosynthetic bacteria found in Bivalvia species from mud volcanoes of the Gulf of Cadiz

Abstract

As in other cold seeps, the dominant bivalves in mud volcanoes from the Gulf of Cadiz are large species belonging to the families Solemyidae (*Acharax* sp., *Petrasma* sp.), Lucinidae (*Lucinoma* sp.), Thyasiridae (*Thyasira vulcolutre*) and Mytilidae (*Bathymodiolus mauritanicus*). $\delta^{13}C$ values measured in solemyid, lucinid and thyasirid specimens support the hypothesis of thiotrophic nutrition whereas, isotopic signatures of *B. mauritanicus* suggest methanotrophic nutrition. The indication by stable isotope analysis that autotrophic bacteria make a substantial contribution to the nutrition of their hosts, led us to investigate the bacterial endosymbionts and their phylogenetic relationships based on comparative 16S rRNA gene sequence analysis. PCR-DGGE analysis and cloning of bacterial 16S rRNA genes confirmed the presence of sulphur-oxidizing symbionts within gill tissues of the studied specimens. Phylogenetic analysis of bacterial 16S rRNA gene sequences demonstrated that most bacteria were related to sulphur-oxidizing endosymbionts known from other deep-sea chemosynthetic environments. Interestingly, despite the indication of the isotopic signature, no known methanotrophic endosymbionts were identified in *Bathymodiolus* specimens, only putative thiotrophic symbionts were found.

This study confirms the occurrence of several chemosynthetic bivalves in the Gulf of Cadiz and further highlights the importance of sulphur-oxidizing symbionts on the trophic ecology of macrobenthic communities in mud volcanoes.

Rodrigues CF, Webster G, Cunha MR & Weightaman AJ (Submitted) Chemosynthetic bacteria found in Bivalvia species from mud volcanoes of the Gulf of Cadiz. Submitted to ***FEMS Microbiology Ecology.***

Chemosynthetic bacteria found in Bivalvia species from mud volcanoes of the Gulf of Cadiz

3.2.1. Introduction

Autotrophic bacteria and marine invertebrate symbioses in deep-sea environments derive all or part of their nutrition from symbiont chemoautotrophy (Fisher 1990; Cavanaugh 1994; Lee *et al*. 1999). Symbiotic associations with thiotrophic (sulphur-oxidizing) and methanotrophic (methane-oxidizing) bacteria occur in a wide range of animal species that live in reducing sediments, such as hydrothermal vents, whale skeletons, sunken wood and cold seeps (reviewed by Duperron 2005; Cavanaugh *et al*. 2006). Sulphur-based symbioses are by far the most commonly reported from natural systems (Distel *et al*. 1988; Imhoff *et al*. 2003; Stewart *et al*. 2005). Bivalves with sulphur-oxidizing gill symbionts are frequently found in environments that are inhospitable to other invertebrates due to low oxygen content and to the presence of free H_2S (reviewed by Southward 1986). Sulphide-oxidizing bacteria are involved in symbioses with many molluscan species, occurring intracellularly in bivalves of the families Vesicomyidae, Lucinidae, Mytilidae and Solemyidae, and mostly extracellularly in bivalves of the family Thyasiridae, These prokaryotic symbionts provide their hosts with an internal source of nutrition and, in turn, the hosts supply the bacteria a stable environment and a regular supply of the inorganic substrates required for chemosynthesis (Cavanaugh *et al*. 1992).

The symbiotic associations of bivalves have been studied with molecular methods and sequences of several host species and their symbionts are published (e.g. Distel *et al*. 1988; Eisen *et al*. 1992; Imhoff *et al*. 2003; Duperron *et al*. 2007). Bivalve symbionts often belong to closely related clades within the *Gammaproteobacteria*. Known sulphur-oxidizing symbionts within the *Gammaproteobacteria* have three distinct life histories: maternally transmitted endosymbionts, environmentally captured endosymbionts, and free living bacteria (Peek *et al*. 1998). Maternally transmitted symbionts are found in solemyid bivalves (Gustafson & Reid 1988; Cary 1994; Krueger *et al*.

1996) and vesicomyid clams (Endow & Ohta 1990; Cary & Giovannonni 1993). Whereas, lucinid clams (Gros *et al*. 1996), thyasirids (Southward 1986) and mytilid mussels (Won *et al*. 2003) contain environmentally captured endosymbionts. However, it is probable that the full symbiont diversity in bivalves has been severaly underestimate. Recently symbionts belonging to other bacterial phyla such Spirochaetes (Duperron *et al*. 2007) or Bacteroidetes (Duperron *et al*. 2008).

The dominant bivalves at mud volcanoes (MV) in the Gulf of Cadiz are large chemosymbiotic species belonging to the families Solemyidae (*Acharax* sp. and *Petrasma* sp.), Lucinidae (*Lucinoma* sp.) (Rodrigues 2009, submitted), Thyasiridae (*Thyasira vulcolutre* Rodrigues & Oliver 2008; see Rodrigues *et al*. 2008) and Mytilidae (*Bathymodiolus mauritanicus* Cosel, 2002; see Génio *et al*. 2008). Solemyid bivalves live in reducing sediments, often in close association with species of the bivalve superfamily Lucinoidea (Reid & Brand 1986). Nutrition in the solemyids is via symbiotic chemoautotrophic bacteria contained in the hypertrophied gills, and their digestive systems vary from being reduced in size though functional to completely absent (reviewed by Métivier & Cosel 1993). *Bathymodiolus* mussels are often among the dominant inhabitants of deep-sea vents and sulphide-hydrocarbon cold seeps worldwide (Pimenov *et al*. 2002; Miyazaki *et al*. 2004; Duperron *et al*. 2005). Mussels may be mixotrophic, feeding on particulate organic matter to supplement symbiont-derived nutrition (Levin 2005). It has been shown that some *Bathymodiolus* species house thiotrophic bacteria, others harbour methanotrophic bacteria, and some harbour both types of symbionts (Fujiwara *et al*. 2000; Duperron *et al*. 2005). Lucinid and thyasirid bivalves occur widely in habitats ranging from subtidal to deep ocean basins (Dufour 2005; Taylor & Glover 2006) and both families include several species known to harbour gill symbionts. Thyasirids share some specialized features with the closely related Lucinidae including: thick gills, reduced palps, a short simple gut and an elongated burrowing foot (Allen 1958). Not all thyasirids possess a symbiosis with sulphide-oxidizing bacteria, for example, 15 of the 26 species examined by Dufour (2005) were asymbiotic and the remaining showed a varying

nutritional dependence on the symbiosis (Dando & Spiro 1993; Dufour & Felbeck 2006).

In this study, the stable isotopic signature of bivalves collected in mud volcanoes from the Gulf of Cadiz were determined to provide information on the nature of the nutritional carbon and sulphur sources. The indication that the activities of autotrophic bacterial make a substantial contribution to the nutrition of these bivalves, led us to examine the diversity of the bacterial symbionts and further investigate their phylogenetic relationship to other bivalve endosymbionts based on comparative analysis of symbiont-specific 16S rRNA genes.

3.2.2. Material and Methods

Study site and bivalve collection. The Gulf of Cadiz, located west of the Straits of Gilbratar, lies at the boundary between the Mediterranean and Atlantic domains (Figure 3.2.1) and encloses a poorly characterized region of the African-Eurasian plate boundary. Submarine mud volcanism occurs throughout the Gulf of Cadiz and presently over 40 mud volcanoes under different geological, physical and biogeochemical settings are known (Pinheiro *et al*. 2003; Van Rensbergen *et al*. 2005; Niemann *et al*. 2006; Hensen *et al*. 2007). The material studied herein was collected during several TTR cruises (IOC-UNESCO) onboard the RV Prof. Logachev and the Microsystems 2007 (NIOZ) cruise onboard the RV Pelagia using mostly a TV-assisted grab or a USNEL box-corer (Table 3.2.1). One to three specimens of each species from different mud volcanoes were dissected and prepared separately for DNA extraction. The soft tissues were removed from the shells, dissected, washed three times in sterile water, and stored -80ºC for endosymbiont characterization studies and stable isotope measurements.

Stable isotope analysis. In the laboratory samples were lyophilized and homogenized in a mortal-and-pestle grinding tool. Following, the powder was separated for ^{13}C, ^{15}N and ^{34}S analyses. The ground sample for carbon analysis was acidified with HCl (1M) until no further bubbling occurred; the sample was re-suspended in distilled water, centrifuged and the supernatant discarded (this procedure was repeated 3 times); finally the sample was dried at 60ºC.

The ground sample for sulphur analysis was re-suspended in distilled water, shaken for 5 min, centrifuged and the supernatant discarded; this procedure was repeated and the sample was dried at 60ºC before being re-ground. The samples were analyzed at ISO-Analytical Laboratory (Cheshire, UK) using the method EA-IRMS (elemental analysis – isotope ratio mass spectrometry).

The isotope compositions are reported relative to standard material and follow the same procedure for all stable isotopic measurements as follows:

$$\delta^{x}E = \left[\left({}^{x}E/{}^{y}E\right)_{sample} / \left({}^{x}E/{}^{y}E\right)_{standard} - 1\right] \times 100 \quad (1)$$

where E is the element analyzed (C, N or S), x is the molecular weight of the heavier isotope, and y the lighter isotope (x=13, 15, 34 and y=12, 14 and 32 for C, N and S, respectively). The standard materials to which the samples are compared are PBD for carbon, air N2 for nitrogen and CTD for sulphur.

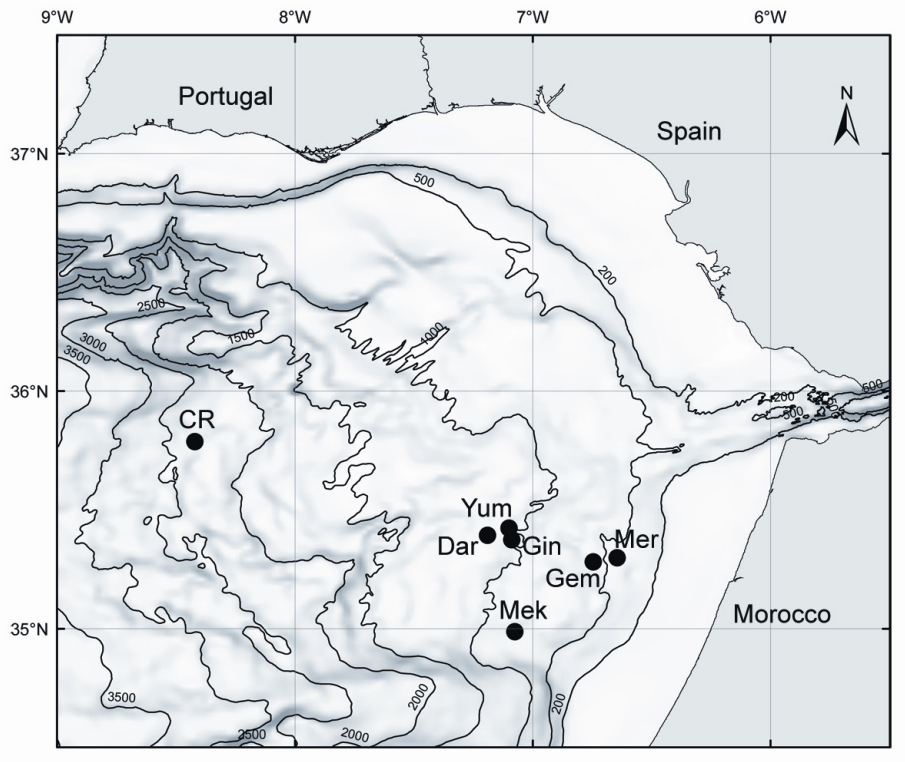


Figure 3.2.1. Location of mud volcanoes in the Gulf of Cadiz where chemosynthetic bivalve species were collected for analysis. CR: Carlos Ribeiro, Dar: Darwin, Gem: Gemini, Gin: Ginsburg, Mek: Meknès, Mer: Mercator, Yum: Yuma.

DNA extraction. DNA was extracted from freeze-dried tissue of bivalves using DNeasy©Blood and Tissue kit (Qiagen) using the manufacturer's protocol with some modifications. In summary, to facilitate DNA extraction,

the tissue was initially placed in lysing matrix E tubes and homogenised for 40s at speed 6 using a FastPrep instrument (QBiogene). Subsequently, samples were incubated at 56ºC until the tissue was completely lysed. The remaining procedure was followed as recommended by the manufacturer. DNA extracts were visualized by standard agarose gel electrophoresis, and the DNA quantified against Hyperladder I DNA marker (Bioline) using the Gene Genus Imaging System (Syngene).

PCR amplification. Bacterial 16S rRNA genes were amplified from tissue DNA samples using the primer combinations 27F-1492R (Lane, 1991) with the following PCR conditions. PCR mixtures contained (total 50µl, molecular grade water) 0.4 pmol μl^{-1} of primers, 1µl of tissue DNA template, 1x reaction buffer (Bioline), 1.5 mM MgCl2, 1.5 U BioTaq DNA polymerase (Bioline), 0.25mM each dNTP, 10 µg bovine serum albumin (BSA). Reaction mixtures were held at 95ºC for 2 min followed by 30 cycles of 94ºC for 30 s, 52ºC for 30 s and 72ºC for 90 s plus 1 s per cycle, with a final extension step of 5 min at 72ºC. PCR products analysed by DGGE were then re-amplified in a nested PCR reaction using the reaction mixture described above without BSA. Bacterial 16S rRNA genes were re-amplified with primers 357FGC-518R (Muyzer *et al*. 1993) at 95ºC for 5 min followed by 10 cycles of 94ºC for 30 s, 55ºC for 30 s, 72ºC for 60 s, and 25 cycles of 92ºC for 30 s, 52ºC for 30 s, 72ºC for 60 s, with a final step of 10 min at 72ºC.

DGGE analysis. DGGE was performed with a DCodeTM Universal Mutation Detection System (Bio-Rad Laboratories). The PCR samples were loaded into 8% (w/v) polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels in a 1x TAE buffer with a denaturing gradient ranging from 30% to 60% (denaturation of 100% corresponds to 7 M urea and 40% (v/v) deionized formamide). Electrophoresis was run for 10 min at 80 V and then 290 min at 200 V in 1x TAE buffer at 60°C. The gel was stained with SYBR-Gold (Molecular Probes). Dominant DGGE bands were excised and sequenced with the 518R primer, and partial bacterial 16S rRNA gene sequences were analyzed to identify sequences with highest sequence identity (Webster *et al*. 2003; Webster *et al*. 2006).

16S rRNA gene libraries. Only products from reactions showing the highest diversity assessed by DGGE and without PCR product in the negative controls were cloned. Each 16S rRNA gene library was constructed from five independent PCR products that were pooled and cleaned with Wizard PCR Preps DNA Purification System (Promega) according to the manufacturer's instructions. Cloning was with pGEM-T Easy (Promega) according to the manufacturer's instructions, using optimised insert:vector ratios and overnight ligation at 4ºC. Libraries were screened by PCR with M13 primers. All 16S rRNA gene clones with verified inserts were randomly selected and sequenced using the ABI 3130xl 16 capillary Genetic Analyzer.

Phylogenetic analysis. Sequence chromatographs were analysed using the Chromas software package version 1.45. (http://www.technelysium.com.au/chromas.html). Partial sequences were checked for chimeras with Chimera Check from the Ribosomal Database Project II (http://rdp8.cme.msu.edu.html/) and their closest relatives identified by NCBI BLAST (http://www.ncbi.nlm.nih.gov). All nucleotide sequences were aligned using ClustalX (Thompson *et al*. 1997) with sequences retrieved from the database. Alignments were edited manually using BioEdit Sequence Alignment Editor version 5.0.9 (Hall 1999) and regions of ambiguous alignment removed. Phylogenetic trees were constructed using neighbour-joining with the Jukes and Cantor correction algorithm in MEGA version 3.1 (Kumar *et al*. 2004). All 16S rRNA gene sequences retrieved during this study were deposited in the database under accession numbers FM213420 to FM213421.

3.2.3.Results and Discussion

Distribution of the bivalve species. Over 40 mud volcanoes (MV) are known in the Gulf of Cadiz and biological sampling was carried out in 18 of them. A number of putative chemosynthetic bivalve species have been found at different mud volcanoes (Figure 3.2.1). Among the Solemyidae *Petrasma* sp. was found mostly in shallower mud volcanoes (Mercator, Gemini, Kidd, Yuma, Ginsburg and Darwin MV) from 358 to 1105m depth while *Acharax* sp showed a deeper bathymetric range (960 to 3902m occurring in Yuma, Ginsburg, Jesuz Baraza, Captain Arutyunov, Carlos Ribeiro and Porto MV)

(Rodrigues 2009, submitted). Solemyids were usually found buried deep in the sediment. Live specimens of *Lucinoma* sp. (Lucinidae) and *Bathymodiolus mauritanicus* (Mytilidae) were recorded from a single mud volcano. *Lucinoma* sp. was collected from a site where active methane bubbling was observed in the crater of Mercator MV (358m) while *B. mauritanicus* occurred along the fissures among the carbonate slabs that cover the crater at Darwin MV (1115m), Finally *Thyasira vulcolutre* (Thyasiridae) was recorded in the craters of Captain Aruryunov MV (1320m), where it reached high densities, and Carlos Ribeiro MV (2200m). Gas hydrates have been collected in these two mud volcanoes that are associated with major tectonic faults and are considered among the most active in the Gulf of Cadiz. The specimens used for endosymbiont characterization in this study and their respective mud volcano locations in the Gulf of Cadiz are listed in Table 3.2.1.

Stable isotope analysis. Analysis of the natural stable isotopic compositions of tissues ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) has been widely used as a means to determine food sources in organisms inhabiting reducing environments (Levin *et al*. 2000; MacAvoy *et al*. 2003; Van Dover *et al*. 2003). In this study different bivalve tissues (gills, foot and mantle) were analyzed separately, but no differences in isotopic values from different tissues were found, suggesting similar degree of carbon flow from bacterial symbiont to the host.

Average carbon isotopic signatures between -52.38±0.98‰ (Bathymodiolus mauritanicus specimens from Darwin MV) and -28.77±0.61‰ (*Acharax* sp. specimen from Ginsburg MV) were measured (Figure 3.2.2). These values are within the range of values reported for bivalves harbouring symbionts (Fisher 1990) and further support the hypothesis for a carbon nutrition source based on bacterial chemoautotrophy.

In hosts with chemosynthetic symbionts, highly negative $\delta^{13}C$ tissue values indicate that their symbionts are methane oxidizers using a carbon source highly depleted in ^{13}C, such as biogenic methane. The $\delta^{13}C$ value determined for mytilids was within the range of methanotrophic mussels (-40 to -70‰) previously found at several hydrocarbon seeps and hydrothermal vents (Kennicutt *et al*. 1992; Conway *et al.* 1994; MacAvoy *et al*. 2005). Tissue carbon isotopic values reflect the relative input of thermogenic and biogenic

methane seepage at a given location (Kennicutt *et al.* 1992) and the values reported here seem to indicate the use of thermogenic methane for mytilid mussels from Darwin MV. Less negative $\delta^{13}C$ values within the range -20 to -40‰ indicate the presence of chemoautotrophic sulphur oxidizers symbionts using a carbon source less depleted in ^{13}C, such as inorganic carbon. Average $\delta^{13}C$ values between -35.57±1.30‰ and -28.77±0.61‰ (Figure 3.2.2) recorded for solemyid, lucinid and thyasirid species in the Gulf of Cadiz are within the range reported for animals with thiotrophic symbionts (Brooks *et al*. 1987).

Average $\delta^{15}N$ values (Figure 3.2.2, upper graph) varied between -3.38±0.26‰ (*Acharax* sp. from Ginsburg MV) and +6.10±0.52‰ (*Petrasma* sp. from Gemini MV). The high values (> 5‰) measured for *Petrasma* specimens from Meknès and Gemini MVs may indicate symbiont absence as suggested by Levin & Michener (2002). In contrast, light (often negative) $\delta^{15}N$ values occurring together with light $\delta^{13}C$ values corroborate the presence of symbionts, which can use local nitrogen sources (Levin & Michener 2002). Differences in nitrogen values between species suggest that they are, either using different chemical species of nitrogen, tapping into different pools of nitrogen, or discriminating differently after acquisition of their nitrogen source (MacAvoy *et al*. 2005).

Average $\delta^{34}S$ results showed a wide range of values from -16.50±3.11‰ (*Petrasma* sp. from Meknès MV) to +16.77±0.20‰ (*Bathymodiolus mauritanicus* from Darwin MV). The $\delta^{34}S$ values (<5‰) in solemyids, lucinids and thyasirids indicate a thiotrophic mode of nutrition (Vetter & Fry 1998). For these species the values between -16.50±3.11‰ (*Petrasma* sp. from Meknès MV) and +1.50±0.92 ‰ (*Thyasira vulcolutre* from Carlos Ribeiro MV) indicate that they rely on sulphides from seeps (Vetter & Fry 1998) since such depleted $\delta^{34}S$ values of tissues indicate a sulphur source other than seawater sulphate (seawater sulphate $\delta^{34}S$ = +21‰; Trust & Fry 1992). Dando & Spiro (1993) concluded that the *Thyasira sarsi* and *Thyasira equalis* nutrional dependence upon their symbionts varied with changing sulphide content in the sediment. Moreover, the highly depleted $\delta^{34}S$ ratios found in *Petrasma* sp. from Mercator and Meknès MV and *Lucinoma* sp. from Mercator MV could be derived from the

utilization of biogenically produced H_2S. On the other hand, the less depleted $\delta^{34}S$ value of the mytilid (*Bathymodiolus mauritanicus*) tissue (+16.77±0.20‰) combined with the highly depleted carbon signature reinforces the evidence that these organisms rely on methane oxidation for their energy production but does not exclude the possibility of some reliance on the assimilatory uptake of inorganic sulphur from seep fluids for the biosynthesis of organic sulphur compounds (Vetter & Fry 1998).

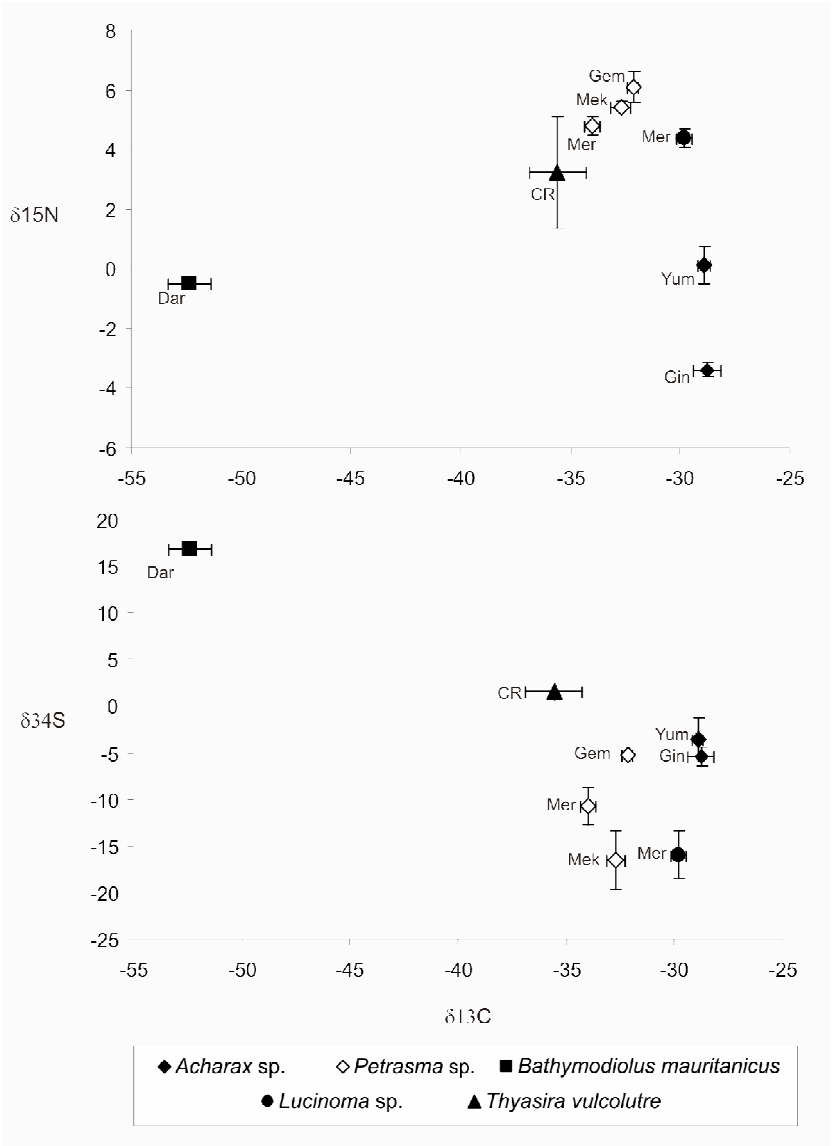


Figure 3.2.2. Dual isotope plot summarizing mean (±1SE) δ13C vs δ15N and δ13C vs δ34S values for bivalves in each mud volcano. CR: Carlos Ribeiro, Dar: Darwin, Gem: Gemini, Gin: Ginsburg, Mek: Meknès, Mer: Mercator, Yum: Yuma.

Since the isotopic fractionation associated with sulphate reduction to H_2S by bacteria is large and variable, the primary sulphur source is often difficult to identify based on $\delta^{34}S$ values alone (Kennicutt *et al*. 1992). Nevertheless, the analysis of stable isotope ratios does support the hypothesis for chemoautotrophic-sourced host carbon nutrition for several species in the Gulf of Cadiz and provided strong evidence to warrant further investigation by molecular methods.

DGGE analysis of bacterial endosymbiont 16S rRNA genes. High molecular weight DNA was extracted from a number of preserved bivalve samples taken from a range of different mud volcanoes. It was observed that genomic DNA extraction from freeze-dried tissue was more consistent after the introduction of an additional step of tissue homogenisation using a FastPrep instrument. It is presumed that that this extra vigorous extraction step facilitated the physical disruption of cellular material and the release of bacterial DNA (Miller *et al*., 1999). Preliminary DNA extractions on ethanol-preserved tissue were not successful and this may have been due to DNA degradation during the extraction procedure similar to that reported for mammalian tissues stored in ethanol (Kilpatrick 2002). The amount of high molecular weight DNA extracted was variable and often related to the size of the specimen. Foot and gill tissues were analysed separately and no bacterial endosymbionts were found in the foot tissue samples analysed.

Denaturing gradient gel electrophoresis (DGGE) profiles of bacterial 16S rRNA genes derived from the gill tissue from several bivalves are shown in Figure 3.2.3 and a summary of the results is presented in Table 3.2.1. The gill symbiont of the *Lucinoma* specimen (Figure 3.2.3, band 1) presented 100% sequence similarity with a 16S rRNA gene from the *Gammaproteobacteria* gill symbiont of *Lucinoma annulata* (Distel *et al.* 1988). This species of *Lucinoma* is only known to occur in the Pacific Ocean whereas other species, taxonomically more similar to the species from the Gulf of Cadiz such as *Lucinoma borealis* and *Lucinoma kazani* are also biogeographically closer. *Lucinoma borealis* is widely distributed in the NE Atlantic (Dando *et al.* 1986) and *L. kazani* was described from one cold seep in the Eastern Mediterranean Sea (Salas & Woodside 2002). These two species are also known to harbour

thiotrophic symbionts (Dando *et al*. 1986; Duperron *et al*. 2007) but DGGE analysis showed no matching phylotype in comparisions between the symbionts from these two species and the ones from *Lucinoma* sp. found at Mercator MV.

Replicate PCR-DGGE analysis of the Mercator MV *Lucinoma* sp. specimen showed sometimes another minor band (not shown in Figure 3.2.3) with 97% sequence similarity to an uncultured Spirochaetes 16S rRNA gene sequence found in microbial mats from Ebro (AY605144). The low abundance of this bacterial phylotype could explain why it was identified sporadically by PCR-DGGE (Mühling *et al.* 2008). Recently, Spirochaetes have been found in a number of other marine invertebrates including a gutless Oligochaeta (Blazejak *et al*. 2005; Ruehland *et al*. 2008), the bivalve *Lucinoma kazani* (Duperron *et al*. 2007) and the hydrothermal vent worm *Alvinella pompejana* (M.A. Combon-Bonavita, unpublished data).

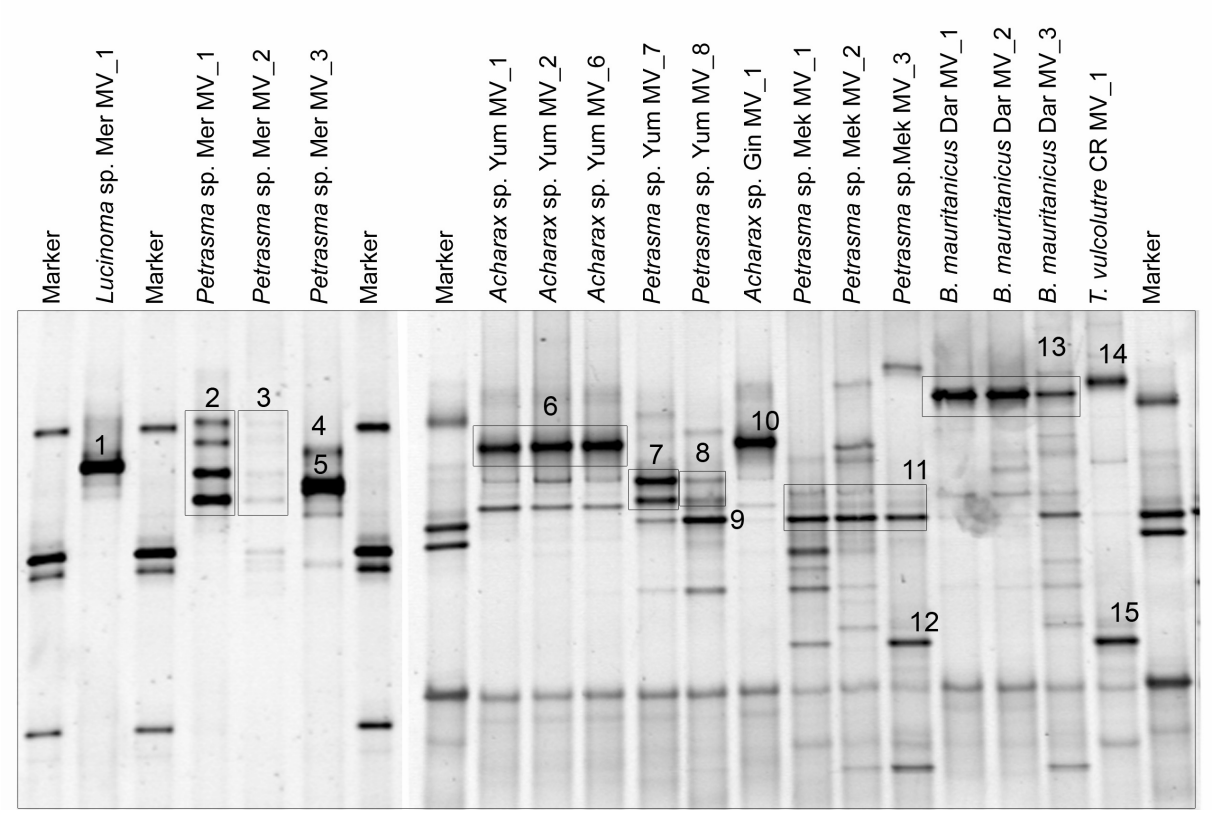


Figure 3.2.3. DGGE profile of bacterial 16S rRNA genes from gills of several chemosynthetic bivalves collected in mud volcanoes from the Gulf of Cadiz. CR: Carlos Ribeiro, Dar: Darwin, Gin: Ginsburg, Mek: Meknès, Mer: Mercator, Yum: Yuma.

A number of *Petrasma* specimens from Mercator, Meknès, Yuma (Figure 3.2.3; Table 3.2.1) and Gemini MVs (Table 3.2.1) were studied. Two specimens of *Petrasma* sp from Mercator MV revealed a group of four bands (labelled bands 2 and 3) all with 99% sequence similarity with the *Solemya velum* symbiont (Eisen *et al*. 1992). Similar banding patterns and sequence similarity to *Solemya velum* symbionts were also found for some *Petrasma* specimens from Yuma MV (group band 7 and 8).

In contrast, the third specimen of *Petrasma* collected at Mercator MV did not reveal the presence of recognised endosymbionts but instead two other phylotypes were observed. Band 4 with 97% sequence similarity to an *Epsilonproteobacteria* clone from a salt marsh (AY711803) and band 5 with 93% sequence similarity to an uncultured *Chlamydia* species (AY159837) found in marine sediment from Lock Duick, Scotland (Freitag & Prosser 2003). These bacteria may be associated with the exterior of the animal, since *Epsilonproteobacteria* are increasingly being recognized as an ecologically significant group of bacteria in deep-sea hydrothermal environments (Nakagawa *et al*. 2005) and cold seeps where they occur has sulphur-oxidizing strains (Takai *et al*. 2005). Alternatively, it is possible that they may be acting as sulphide detoxifiers in the same way as suggested for an *Epsilonproteobacteria* phylotype found in two endemic hydrothermal vent fauna (Campbell *et al.* 2006). The presence of *Chlamydia*-like bacteria may be indicative of the occurrence of parasitism, similar to the *Chlamydia*-like inclusions that were described from digestive tissue of Bathymodiolus heckerae from Blake Ridge (Ward *et al*. 2004). Parasitic infections can impair growth, reproduction, competitive ability, stress tolerance and survival of host species (reviewed in Ward *et al.* 2004). It should be noted that from the three bivalve specimens analysed from Mercator MV, only the smallest one revealed the presence of this bacterial phylotype.

Table 3.2.1. Summary of the presence of endosymbionts and type of chemolithotrophy in several bivalves collected on mud volcanoes from the Gulf of Cadiz. ND - endosymbionts not detected.

Species	Mud volcano cruise, station	Latitude/ Longitude (water depth m)	Number of specimens collected	Evidence of chemolithotrophy by stable isotope analysis	Endosymbionts	Nearest 16S rRNA gene sequence match (sequence similarity)	Phylogenetic group
Acharax sp.	Ginsburg MV TTR16, AT607Gr	35º22.677N/ 07º04.979W 983	1	Thiotrophic	Present	*Acharax johnsoni* endosymbiont (98%)	*Gammaproteobacteria*
	Yuma MV TTR16, AT604Gr	35º25.820N/ 07º06.330W 1030	3	Thiotrophic	Present	*Acharax johnsoni* endosymbiont (98%)	*Gammaproteobacteria*
Petrasma sp.	Mercator MV TTR15, AT569Gr	35º17.917N 06º38.717W 358	3	Thiotrophic	Present	*Solemya velum* endosymbiont (99%)	*Gammaproteobacteria*
	Meknès MV TTR15, AT586Gr	34º59.146N 07º04.380W 701	3	Thiotrophic	ND	Other bacteria	Several
	Yuma MV TTR16, AT604Gr	35º25.820N/ 07º06.330W 1030	2	Thiotrophic	Present	*Solemya velum* endosymbiont (99%)	*Gammaproteobacteria*
	Gemini MV Microsystems2007 M2007_10	35º16.900N 06º45.350W 418	2	Thiotrophic	Present	*Solemya velum* endosymbiont (96%)	*Gammaproteobacteria*
Lucinoma sp.	Mercator MV TTR15, AT569Gr	35º17.917N 06º38.717W 358	1	Thiotrophic	Present	*Lucinoma annulata* endosymbiont (100%)	*Gammaproteobacteria*
Bathymodiolus mauritanicus	Darwin MV TTR16, AT608Gr	35º23.531N 07º11.475W 1115	3	Methanotrophic	Present	*Bathymodiolus puteoserpentis* thioautotrophic endosymbiont (100%)	*Gammaproteobacteria*
Thyasira vulcolutre	Carlos Ribeiro MV TTR16, AT615Gr	35º47.238N 08º25.272W 2200	2	Thiotrophic	Present	Uncultured bacterial clone name (88%)	*Alphaproteobacteria*

PCR-DGGE analysis of bacterial 16S rRNA genes from Meknès MV showed the absence of known symbiont bacteria which may be indicative of sparseness of symbionts in these specimens. Sequence analysis of DGGE bands from the gills of all Meknès MV *Petrasma* specimens revealed no known symbiont phylotypes (Figure 3.2.3). A phylotype (band 11) with 97% similarity to an uncultured *Gammaproteobacterium* (EF202342) found in the bacterioplankton from the Gulf of Maine (Stepanauskas & Sieracki 2007). The third specimen analysed revealed a phylotype (band 12) with 97% sequence similarity to *Bradyrhizobium* sp.L22 (EF436497), a known symbiotic nitrogen fixer found in the roots nodules of Acacia longifolia. Symbioses of *Solemya reidi* exhibited nitrate and nitrite respiration under anoxic conditions but also occurred when the whole animal was under oxic conditions. The switch to such anaerobic respiration would facilitate the conservation of oxygen for host metabolism during periods of low oxygen tensions (Wilmot & Vetter 1992). At Meknès MV sulphate reduction and consequently production of sulphide seems to be low (Webster *et al*. unpublished data). This observation coupled with the evidence of symbiont absence from the observed high $\delta^{15}N$ values (Figure 3.2.2) may indicate that these specimens do not rely only on chemoautotrophic nutrition and/or that symbiont density may vary in these bivalve species.

As noted above rare bacterial species within a total prokaryotic population may not always be detected by PCR-DGGE (Muyzer *et al.* 1993). High sequence similarity (> 98%) was found between the endosymbionts of *Acharax johnsoni* (Sahling *et al*. 2003) and symbionts from *Acharax* specimens collected at Yuma and Ginsburg MV where the bands migrated to the same position on DGGE gel (bands 6 and 10 respectively; Figure 3.2.3).

Despite the evidence for methanotrophic nutrition provided by stable isotope results, the analysis of mytilid specimens presented a 100% sequence similarity with *Bathymodiolus puteoserpentis* thioautotrophic symbionts (bands 13; Figure 3.2.3) (Won *et al.* 2003). An explanation for the highly depleted ^{13}C signatures of *B. mauritanicus* is that their thiotrophic symbionts incorporate inorganic carbon from unusually light CO_2 produced by sediment associated metanotrophs but there are no data available either on the presence of these sediment prokaryotes or on the isotopic signatures of methane from Darwin

MV. Athough methane cycling is a major biogeochemical process in other mud volcanoes from the Gulf of Cadiz (Niemann *et al.* 2006). An alternative hypothesis is the occurrence of a dual symbiosis with sulphur and methane oxidizers in *B. mauritanicus*, as it is known to occur in *Bathymodiolus azoricus* and *B. puteoserpentis* (Duperron *et al*. 2006). However, this implies that we were not able to detect the methanotrophic symbiont in the three specimens analysed, and further investigation needs to be done using FISH and/or TEM techniques to detect the presence of significant amounts of methanotrophic oxidizers in the gills of *B. mauritanicus*. In addition the DGGE profile also shows a number of other unidentified bands.

DGGE results for *Thyasira vulculotre* revealed a novel phylotype (band 15) with only 88% sequence similarity with an uncultured *Alphaproteobacteria* phylotype from hydrothermal sediment collected at Mid-Atlantic Ridge (Lopez-Garcia *et al*. 2003). Another band (14) was present in the DGGE results and revealed an 85% sequence similarity to *Thiorhodococcus kakinadensis* (a purple sulphur bacteria) and 85% also similar to the ammonia oxidiser *Nitrosococcus oceani*.

Because only a fraction of thyasirid species are symbiotic, and the symbionts are mostly extracellular, this group may be representative of early stages in the evolution of bacterium-bivalve symbiosis (Dufour 2005). Consequently, thyasirid symbionts that have been phylogenetically characterized fall into separate clades of *Gammaproteobacteria*, rather than a single clade as in most other chemosymbiotic bivalves (Imhoff *et al.* 2003).

Analysis of 16S rRNA gene libraries from MV bivalve specimens. The results showed that the putative symbionts phylotypes from *Acharax* sp. (from Yuma and Ginsburg MV), *Petrasma* sp. (from Gemini, Mercator and Yuma MV), *Lucinoma* sp. (from Mercator MV) and *Bathymodiolus mauritanicus* (from Darwin MV) were assigned within the *Gammaproteobacteria* clade (Figure 3.2.4a), in which many thiotrophic and methanotrophic symbionts of marine invertebrates are included. In fact, the association between endosymbionts and host animals can be highly specific. Since none of these phylotypes show a close relationship to free-living sulphur bacteria, it can be concluded that

symbiotic and free-living sulphur bacteria represented separate lineages that have undergone a long period of divergence (Imhoff *et al*. 2003).

The occurrence of the almost identical symbiont phylotypes in specimens of *Acharax* sp. from geographically very distant localities (*Acharax johnsoni* occurs in Pacific Sea) is quite remarkable. It indicates that the symbionts of *Acharax* are relatively modern descendants of a much more ancient phylogenetic line. The large phylogenetic distances between symbionts of different Solemyidae species support their ancient evolutionary history with the solemyid genera *Solemya* and *Acharax* having a particularly deep branching point compared to other clusters of symbiotic bacteria (Imhoff *et al.* 2003).

Thyasira vulcolutre clones showed some relation with a sub-cuticular bacteria present in the ophiuroid *Ophiopholis aculeata* (Burnett unpublished), with a scleractinian coral symbiont (Rohwer unpublished) and also with a sponge symbiont. One clone from *Lucinoma* sp. was related to symbionts found in deep-sea octocorals (Penn *et al.* 2006) known to harbour several bacteria groups. These symbionts showed some relation with Spirochaetes strains that occur in oligochaetes but are not necessary symbionts (e.g. *Lucinoma kazani*, Duperron *et al.* 2006). Clones belonging to *Betaproteobacteria*, *Epsilonproteobacteria* and *Actinobacteria* were also found in *Petrasma* sp. specimens.

Chemosynthetic bacteria found in bivalves from Gulf of Cadiz – an overview. Except for maybe *Bathymodiolus mauritanicus* molecular analysis of the bacterial symbionts agreed with stable isotope data and in all cases 16S rRNS gene libraries agreed with PCR-DGGE analysis, which suggested the occurrence of a main thiotrophic symbiont for all the species analysed. Therefore, the Gulf of Cadiz MV endosymbiotic community is similar to other vent and seeps symbioses in that thiotrophy or sulphur oxidation is the dominant bacterial chemoautotrophic activity (Fisher 1990). Energy utilized by the symbiont is derived from the oxidation of reduced sulphur (H_2S, thiosulphate, etc) and carbon is derived from dissolved inorganic carbon (DIC) from seawater (Kennicut *et al*. 1992).

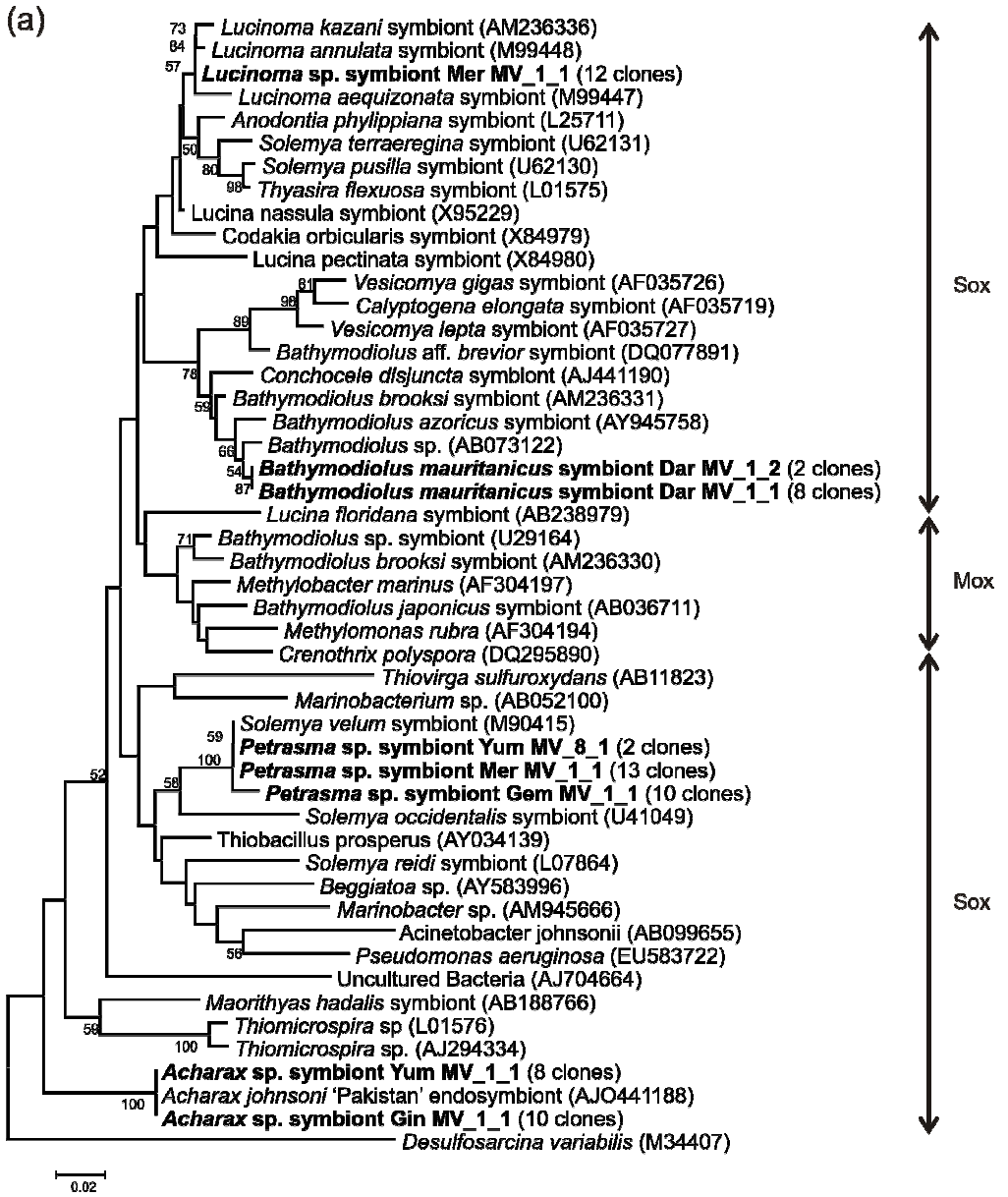
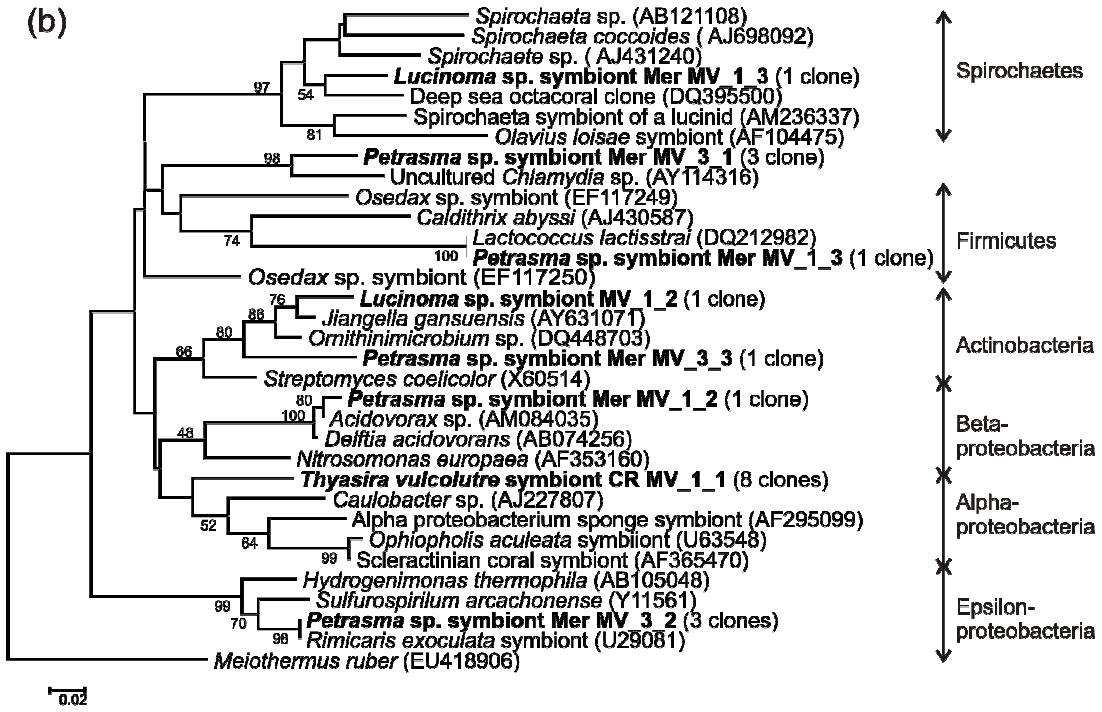


Figure 3.2.4. Phylogenetic relationship, based on Neighour-Joining (Jukes Cantor Model) analyses of bacterial 16S rRNA gene sequences retrieved from the gills of some bivalves from the Gulf of Cadiz. Bootstrap supports values over 50% (1000 maximum-likehood replicates) are shown. Scale bar=0.02 estimated substitutions. (a) *Gammaproteobacteria*, Sox – Sulphur-oxidizing bacteria, Mox – Methano-oxidizing bacteria; (b) other Bacteria. CR: Carlos Ribeiro, Dar: Darwin, Gem: Gemini, Gin: Ginsburg, Mek: Meknès, Mer: Mercator, Yum: Yuma.



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The solemyids *Acharax* sp. and *Petrasma* sp. have a widespread distribution in the Gulf of Cadiz. Despite the range of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values suggesting different uptakes and fractionation processes the symbionts are species highly specific. The absence of symbionts in Petrasma specimens collected in Meknès MV confirms the status of non-obligatory symbiosis for these species. Of the five species studied in the Gulf of Cadiz, *Acharax* sp. and *Petrasma* sp. are the only hosts that contain maternally transmitted endosymbionts and the apparently worldwide distribution of these symbionts may result from an ancestral distribution of their primordial hosts.

Table 3.2.2. Bacterial 16S rRNA gene libraries obtained from bivalve gill tissue of several species in the Gulf of Cadiz.

Species	Mud volcano	Number of clones	Representative clone	Nearest 16S rRNA gene sequence match, accession number (sequence similarity)
Acharax sp.	Ginsburg	10	*Acharax* sp. endosymbiont Gin MV_1	*Acharax johnsonni* endosymbiont, AJ0441188 number (98%)
	Yuma	8	*Acharax* sp. endosymbiont Yum MV_1	*Acharax johnsonni* endosymbiont, AJ0441188 (98%)
Petrasma sp	Gemini	10	*Petrasma* sp. endosymbiont Gem MV_1	*Solemya velum* symbiont, M90415 (96%)
	Mercator	13	*Petrasma* sp. endosymbiont Mer MV 1_1	*Solemya velum* symbiont, M90415 (98%)
		1	*Petrasma* sp. endosymbiont Mer MV_1_2	Uncultured *Acidovorax* sp., EU705182 (98%)
		1	*Petrasms* sp. endosymbiont Mer MV_1_3	*Lactococcus lactisstrai*, DQ212982 (99%)
	Mercator	3	Petrasma sp. endosymbiont Mer MV_3_1	Uncultured *Chlamydia* sp, AY114316 (94%)
		3	Petrasma sp. endosymbiont Mer MV_3_2	Uncultured epsilon proteobacterium, DQ112520 (96%)
		1	*Petrasma* sp. endosymbiont Mer MV_3_3	Uncultured *actinobacterium*, AY770699 (97%)
	Yuma	2	*Petrasma* sp. endosymbiont Yum MV_8	*Solemya velum* symbiont, M90415 (98%)
Lucinoma sp.	Mercator	12	*Lucinoma* sp. endosymbiont Mer MV_1_1	*Lucina floridana* symbiont, L25707 (97%)
		1	*Lucinoma* sp. endosymbiont Mer MV_1_2	*Jiangella gansuensis,* AY631071 (92 %)
		1	*Lucinoma* sp. endosymbiont Mer MV_1_3	Uncultured organism clone ctg_CGOAB15, DQ395500 (88%)
Bathymodiolus mauritanicus	Darwin	8	*Bathymodiolus mauritanicus* endosymbiont Dar MV_1_1	*Bathymodiolus brooksi* thiotrophic endosymbiont, AM236331 (98%)
		2	*Bathymodiolus mauritanicus* endosymbiont Dar MV_1_2	Uncultured *gamma proteobacterium*, AB112455 (96%)
Thyasira vulcolutre	Carlos Ribeiro	8	*Thyasira vulcolutre* endosymbiont CR MV_1	Uncultured *alpha proteobacterium*, AF424269 (90%)

The thioautotrophic metabolism of solemyid symbioses depends on the availability of sulphide in the marine sediments in which these associations occur (Stewart & Cavanaugh 2006). According to Felbeck (1983) the gill symbionts provide several benefits to the clam; the bacteria may detoxify H_2S, a compound that is extremely toxic due to its effects on metaloenzymes and other metalocompounds, the sulphur bacteria may use sulphide as an energy source for the synthesis of ATP and reducing power (NAD(P)H), and these compounds may be used to drive net CO_2 fixation via the Calvin-Benson cycle and nitrate reduction (Anderson *et al.* 1987; Wilmot & Vetter 1992, Stewart & Cavanaugh 2006).

Environmental transmitted symbionts were identified in *Lucinoma* sp., *Thyasira vulcolutre* and *Bathymodiolus mauritanicus*. Like in *Lucinoma kazani* the occurrence of a Spirochaetes is reported for our *Lucinoma* specimen. All the five species analysed showed the presence of *Gammaproteobacteria* symbionts and *Thyasira vulcolutre* also showed the presence of putative endosymbionts belonged to the *Alphaproteobacteria*. The stable isotope values for the mytilid *Bathymodiolus mauritanicus* found in Darwin MV suggested the occurrence of methanotrophic bacteria, but these results were not confirmed by molecular studies.

This study confirmed the occurrence of several chemosynthetic species in the Gulf of Cadiz, with the dominance of sulphur-oxidizing symbionts. The possible benefits to the partners in chemosynthetic symbioses are of varying importance in these symbioses and more studies need to be done to understand the role of these symbionts for these species. FISH techniques must be applied to confirm the symbiont presence, in particularly for *Bathymodiouls mauritanicus* specimens where global results suggest the occurrence of dual symbiosis.

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Chapter 3.3.Microbial diversity in Frenulata (Polychaeta: Siboglinidae) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic)

Abstract

In this study we used molecular tools to investigate the diversity of symbiotic bacteria associated with several Frenulata species discovered in mud volcanoes from the Gulf of Cadiz. A large diversity is clear but no patterns were observed between species, depth or mud volcano suggesting no selection for specific symbionts.

Rodrigues CF, Hilário A, Webster G, Cunha MR & Weightman AJ (in preparation) Microbial diversity in Frenulata (Polychaeta: Siboglinidae) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic). For submission as a short paper to ***Applied and Environmental Microbiology***

Microbial diversity in Frenulata (Polychaeta: Siboglinidae) from the mud volcanoes of the Gulf of Cadiz (NE Atlantic)

3.3.1.Short Paper

Chemosynthetic-based communities, often dominated by frenulates, have been found in several mud volcanoes (MVs) in the Gulf of Cadiz (GoC) (Cunha *et al.* 2001; Rodrigues & Cunha 2005). Molecular studies show that there at least 13 different species belonging to 6 genera (Hilário *et al*. submitted) including *Siboglinum*, *Lamellisabella*, *Polybrachia*, *Spirobrachia* and *Bobmarleya* (Hilário & Cunha 2008).

Thiotrophic bacteria are found as intracellular endosymbionts in the majority of Siboglinidae (Bright & Giere 2005). Adult siboglinids lack a digestive tract and live in an obligatory association with endosymbiotic bacteria (Cavanaugh *et al.* 1981; Felbeck 1981; Southward 1982; Southward *et al.* 1986), epitomizing the perfect animal-microbe symbiosis. The endosymbionts are in most cases sulphide oxidizing bacteria that generate organic material from inorganic compounds delivered to the trophosome by the host vascular and celomic systems (Arp & Childress 1981; Childress *et al*. 1984). The exceptions are *Siboglinum poseidoni*, a frenulate species initially described from methane-rich sediments of the central Skagerrak (Schmaljohann & Flügel 1987) that harbours methane-oxidizing endosymbionts, and the genus *Osedax*, which relies on intracellular heterotrophic microbes that are known to metabolize complex carbon compounds (Goffredi *et al*. 2005). The host worms supply endosymbionts via the circulatory system with oxygen, carbon dioxide, and hydrogen sulphide from seawater (Felbeck 1981). The endosymbiotic bacteria oxidize sulphides and methane, synthesize organic compounds, and provide nutrients and metabolic energy to the host worm (Cavanaugh 1985).

Previous studies revealed that siboglinid endosymbionts belong to several phylotypes of the *Gammaproteobacteria* (Di Meo *et al.* 2000; Nelson & Fisher 2000; McMullin *et al*. 2003; Kubota *et al*. 2007; Vrijenhoek *et al*. 2007; Lösekan *et al*. 2008; Thornhill, *et al*. 2008), but these symbiont groups do not

seem to correlate with specific host classification, suggesting acquisition of local and habitat-specific endosymbionts. Such an "opportunistic environmental acquisition" of symbionts is hypothesized to be an ecologically flexible strategy that allows an invertebrate host to incorporate locally adapted microbial strains (Won *et al.* 2003).

Here we examine the microbial diversity in several specimens of Frenulata from several MVs in the GoC (Figure 3.3.1, Table 3.3.1).

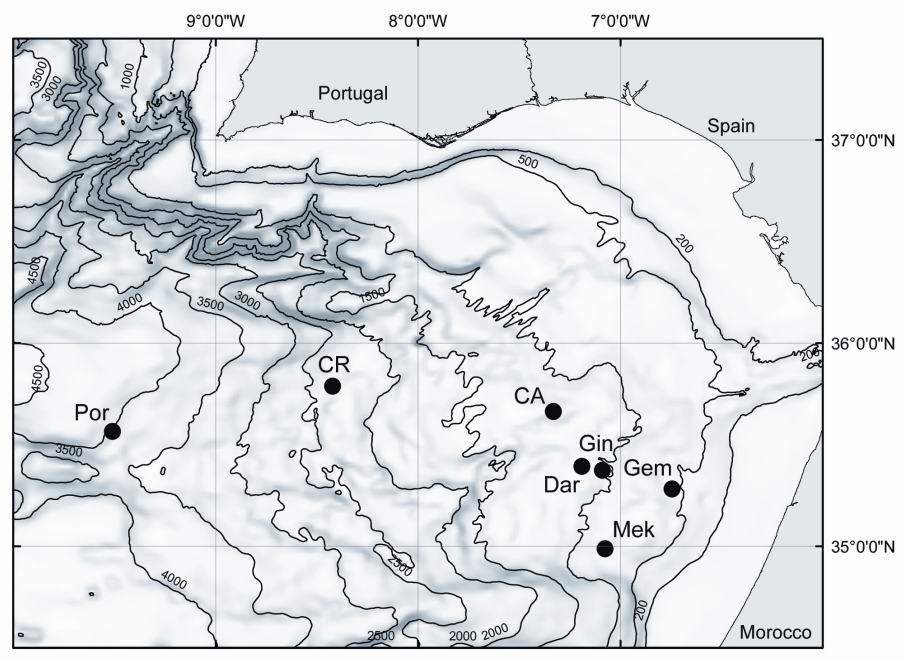

Figure 3.3.1. Study area. Mud volcanoes: CA, Captain Arutyunov; CR, Carlos Ribeiro; Dar, Darwin, Gem, Gemini; Gin, Ginsburg; Mek, Mèknes; Por, Porto

The animals were fixed in ethanol and dissected after preservation out of their tubes. A portion of the trunk of each animal was used for DNA extractions. DNA was extracted using the DNeasy® Blood and Tissue kit (QIAGEN). Bacterial 16S rRNA genes were amplified, and Denaturing Gradient Gel Electrophoresis (DGGE) was performed as previously described (Webster *et al*. 2006). Dominant DGGE bands were excised and sequenced (O'Sullivan *et al.* 2008), and partial bacterial 16S rRNA gene sequences were analyzed using the NCBI nucleotide blast program (http://www.ncbi.nlm.nih.gov/) to identify sequences with highest identity. A set of functional gene PCR primers

for particulate methane mono-oxygenase alpha (*pmoA*) (Holmes *et al*. 1995) were also used. Clone libraries were constructed and sequence chromatographs were analysed following Webster *et al.* (2006). Guide trees were constructed using Neighbour-joining with the Jukes and Cantor correction algorithm in MEGA version 3.1 (Kumar *et al*. 2004).

Table 3.3.1. Sample stations and frenulate species used in this study listed by depth of mud volcano.

Mud Volcano (MV)	**Cruise/ Station**	**Latitude/ Longitude**	**Depth (m)**	**Species (specimen code)**
Gemini MV	M2006	35°16.830'N	432	*Siboglinum* sp.5 (16)
	M2006-10	06°45.54'W		
Meknes MV	MSM01.03	34°59.100'N	695	*Siboglinum* sp.4 (15)
	Stn.319	07°4,439W		
Ginsburg MV	TTR16	35°22.677'N	983	*Siboglinum* sp.2 (12)
	AT607GR	07°4.979'W		
Darwin MV	TTR16	35°23.531'N	1115	*Siboglinum* sp.3 (13)
	AT608GR	07°11.475'W		*Siboglinum* sp.2 (14)
Captain Arutyunov MV	MSM01.03	35°39.668'N	1320	*Siboglinum* sp.1 (9)
	Stn.190	07°19.970'W		*Siboglinum* cf. *poseidoni* (10)
Carlos Ribeiro MV	TTR16	35°47.238'N	2200	*Bobmarleya gadensis* (17)
	AT615GR	08°25.272'W		
Porto MV	TTR16	35°33.773'N	3902	*Spirobrachia tripeira* (1, 3, 4, 5)
	AT622GR	08°30.416'W		*Lamellisabella denticulata (*2)
				Undetermined juvenil (6, 7, 8)
				Undetermined *genus*. (11)

The general characterization using DGGE of the microbial diversity in the trunk allowed in some cases the symbiont identification. As in other studies (Campbell & Cary 2001; Kubota *et al*. 2007) the strongest band obtained was considered to represent the dominant bacterial (endosymbiont) species in each specimen. A complementary study was carried out using clone libraries. Although a few clones were sequenced per host, it is possible that bacterial lineages went mostly undetected in this survey.

Multiple bands in the DGGE profile (Figure 3.2.2, Table 3.2.2) indicate the presence of several bacterial phylotyes in the trophosome of a single host.

Mixed symbiont communities have already been found in vestimentiferans (Fisher & Childress 1984; de Burgh *et al.* 1989). Most of the phylotypes found here belonged to *Gammaproteobacteria*, but also *Alpha-, Epsilon-, Deltaproteobacteria, Actinobacteria, Bacteroidetes* and candidate division TM7 were also found. In some cases the same bacterial phylotype was found in different Frenulata species from different MVs. In addition to the diverse range of bacteria identified, the environmental transmission found for other species (Nussbaumer *et al*. 2006) is also in evidence from the DGGE results. For example, the major bacterial phylotype found in the adult of the undetermined genus from Porto MV (band 8) was absent in the three juveniles.

The DGGE results show a clear difference between the main phylotypes of the two specimens of *Siboglinum* from Captain Arutyunov MV. The trophosome symbiont of specimen 9 presented similarity with an uncultured *Piscirickettsiaceae* (Liao *et al*. 2007), a group that includes the known free-living sulphur-oxidizing *Thiomicrospira* species, and was identified as a symbiont phylotype on a lucinidae species (Ball *et al.* unpublished); specimen 10 presented similarity with *Methylomonas rubra* (accession number AF304194) a known methanotrophic bacteria. However, the results of clone library sequencing also indicate the presence of a methonotrophic symbiont in specimen 9. Aerobic methanotrophic bacteria, including methylotrophs, can utilize methane and/or a variety of C1 compounds, such as methanol and methylated amines, as sole carbon and energy sources (Hanson & Hanson 1996). These organisms are phylogenetically affiliated with two distinct lineages of proteobacteria, the *Gamma-proteobacteria* (type I methanotrophs) and the *Alpha-proteobacteria* (type II methanotrophs), and are widespread in marine environments (Wang *et al*. 2003; Inagaki *et al*. 2004). The second specimen (*Siboglinum poseidoni*) was the only frenulate to give a positive result with the *pmoA* primers (88% sequence similarity with an uncultured bacterial p*moA* gene (accession number AB176937) from methane seep habitats (Inagaki *et al.* 2004). Although methanotrophic bacteria have previously been identified from the trophosome of this species (Schmaljohann & Flügel 1987) our results provide the first molecular evidence that *Siboglinum poseidoni* harbours methanotrophic symbionts.

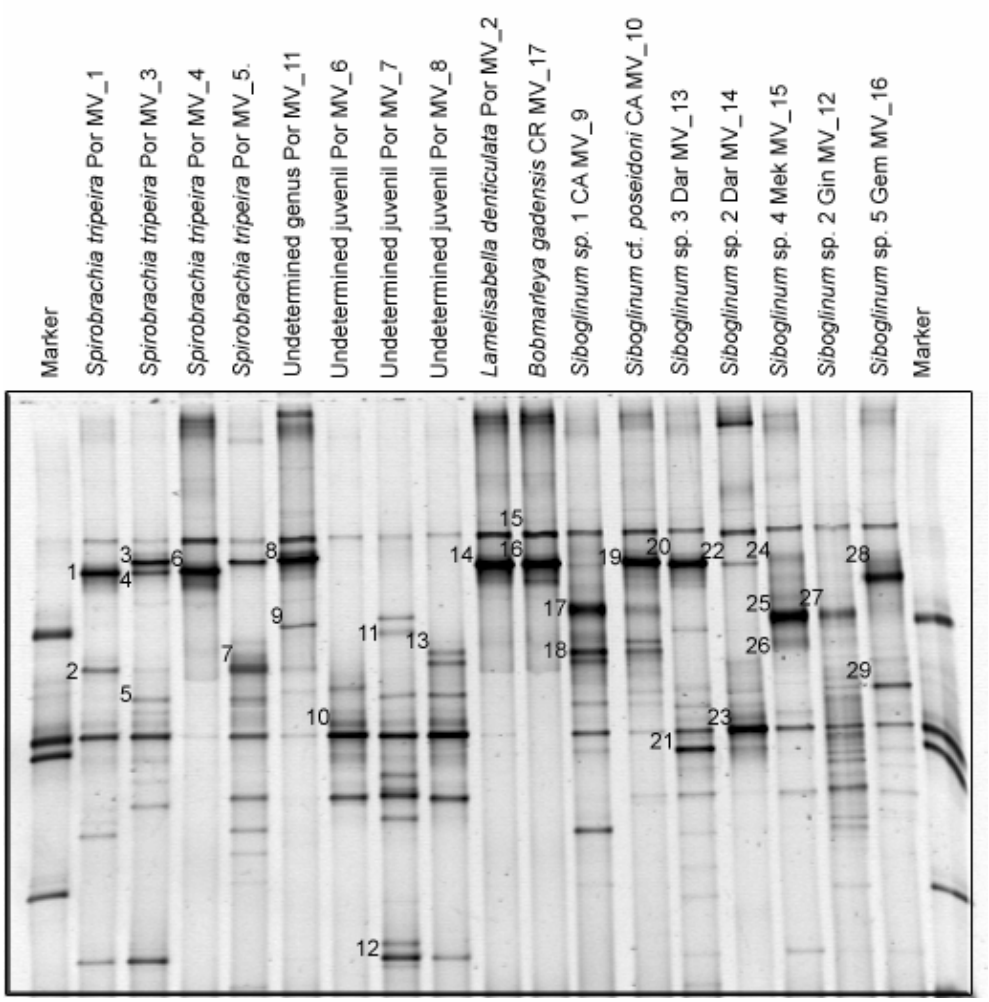

Figure 3.3.2. DGGE profile of bacterial 16S rRNA gene from trophosome of several chemosynthetic Frenulata collected in mud volcanoes from the Gulf of Cadiz.

Several sequences presented a high similarity with the endosymbiont of *Oligobrachia haakonmosbiensis* (Lösekann *et al*. 2008). The sequence obtained from the main DGGE band of *Siboglinum* sp.4 from Meknès MV was 96% similar to the endosymbiont of *Oligobrachia mashikoi* (Kubota *et al*. 2007). Based on the presence of genes for RuBisCO, Kimura *et al.* (2003b) suggested that the *O. mashikoi* endosymbionts might be methanotrophic. However, no *pmoA* genes were detected in *O. mashikoi* (Deguchi *et al.* 2007) or in *Siboglinum* sp.4, and the phylogenetic analyses of the clone libraries for *Siboglinum* sp.4 suggest that these endosymbionts are sulphide- rather than methane-oxidizers.

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Table 3.3.2. DGGE results from frenulate trophosome tissue of several species in the Gulf of Cadiz. MV, Mud volcano: Por, Porto; CR, Carlos Ribeiro, CA, Captain Arutyunov; Dar, Darwin; Mek: Meknès; Gin, Ginsburg; Gem, Gemini.

Species (Specimen code)	MV	DGGE band	Nearest 16S rRNA gene sequence match	Bacterial phylogenetic group	Sequence similarity
Spirobrachia tripeira (1)	Por	1	Endosymbiont of *Oligobrachia haakonmosbiensis* (AM883178)	*Gammaproteobacteria*	98%
Spirobrachia tripeira (1)		2	Campylobacteraceae bacterium isolate ACEMC 7-6 (FM163102)	*Epsilonproteobacteria*	98%
Spirobrachia tripeira (3)		3	Uncultured bacterium clone B16 (EU107481) from hair of *Shinkaia crosnieri*, Okinawa Trench	*Gammaproteobacteria*	97%
Spirobrachia tripeira (3)		4	Endosymbiont of *Oligobrachia haakonmosbiensis* (AM883178)	*Gammaproteobacteria*	95%
Spirobrachia tripeira (3)		5	Uncultured bacterium clone DPEU18 (EF127670) from Antarctic surface water.	*Actinobacteria*	
Spirobrachia tripeira (4)		6	Uncultured bacterium clone pIR3BE07 (AY354135) associated with a carbonate-rich metalliferous sediment ,Rainbow vent field, Mid-Atlantic Ridge	*Gammaproteobacteria*	96%
Spirobrachia tripeira (5)		7	Uncultured bacterium clone VH-FL6-38 (EF379678) from seawater, Victoria Harbor, Hong Kong	*Epsilonproteobacteria*	96%
Undetermined genus (11)		8	Uncultured bacterium clone BJS8R-085 (AB239069) from deep-sea cold seep sediment, Japan Sea	*Gammaproteobacteria*	98%
Undetermined genus (11)		9	Uncultured bacterium clone B16 (EU107481) from hair of *Shinkaia crosnieri*, Okinawa Trench	*Gammaproteobacteria*	99%
Undetermined Juvenil (6)		10	Uncultured bacterium clone 144 (EF188407) from Altamira Cave	Candidate division TM7	94%
Undetermined Juvenil NID (7)		11	Uncultured bacterium clone 1.46 (AM991362) from Arctic lake water	*Bacteroidetes*	80%
Undetermined Juvenil NID (7)		12	Uncultured bacterium clone DPEU18 (EF127670) from Antarctic surface water.	*Actinobacteria*	98%
Undetermined Juvenil NID (8)		13	Endosymbiont 'Oregon 71' of *Acharax johnsoni* (AJ441185)	*Gammaproteobacteria*	90%
Lamelisabella denticulata (2)		14	Uncultured bacterium from Shinkaia crosnieri (vent galatheid crab) (AB476228)	*Gammaproteobacteria*	96%

Species (Specimen code)	MV	DGGE band	Nearest 16S rRNA gene sequence match	Bacterial phylogenetic group	Sequence similarity
Bobmarleya gadensis (17)	CR	15	Uncultured bacterium clone pIR3BE07 (AY354135) associated with a carbonate-rich metalliferous sediment, Rainbow vent field, Mid-Atlantic Ridge	*Gammaproteobacteria*	97%
Bobmarleya gadensis (17)		16	Endosymbiont of *Oligobrachia haakonmosbiensis* (AM883179)	*Gammaproteobacteria*	95%
Siboglinum sp.1 (9)	CA	17	Uncultured bacterium clone DS021 (DQ234105) from mangrove sediment, Danshui river, Taiwan	*Gammaproteobacteria*	93%
Siboglinum sp.1 (9)		18	Endosymbiont 'Oregon 56' of *Acharax johnsoni* (AJ441185)	*Gammaproteobacteria*	100%
Siboglinum cf. *poseidoni* (10)		19	*Methylomonas rubra* (AF304194)	*Gammaproteobacteria*	95%
Siboglinum sp.3 (13)	Dar	20	Endosymbiont of *Oligobrachia haakonmosbiensis* (AM883179)	*Gammaproteobacteria*	97%
Siboglinum sp.3 (13)		21	Uncultured bacterium clone pLM5B-7 (AB247832) from a hydrothermal vent chimney, Mariner hydrothermal field, Lau Basin	*Deltaproteobacteria*	91%
Siboglinum sp.2 (14)		22	Uncultured bacterium clone ELB19-197 (DQ015828) from Antarctic lake water	*Alphaproteobacteria*	94%
Siboglinum sp.2 (14)		23	*Anaplasma platys* (EF139459)	*Alphaproteobacteria*	94%
Siboglinum sp.4 (15)	Mek	24	Uncultured bacterium clone pIR3BE07 (AY354135) associated with a carbonate-rich metalliferous sediment, ,Rainbow vent field, Mid-Atlantic Ridge	*Gammaproteobacteria*	98%
Siboglinum sp.4 (15)		25 and 26	Endosymbiont *of Oligobrachia mashikoi* (AB271122)	*Gammaproteobacteria*	96%
Siboglinum sp.2 (12)	Gin	27	Uncultured gamma proteobacterium (FN397817) from sediment from the Gulf of Cadiz	*Gammaproteobacteria*	95%
Siboglinum sp.5 (16)	Gem	28	Uncultured bacterium clone pIR3BE07 (AY354135) associated with a carbonate-rich metalliferous sediment ,Rainbow vent field, Mid-Atlantic Ridge	*Gammaproteobacteria*	98%
Siboglinum sp.5 (16)		29	Uncultured bacterium clone 1P-2-G22 (EU705047) from a cleanroom floor	*Gammaproteobacteria*	86%

The presence of different symbiotic phylotypes in the same species, from the same site, as found in the *Spirobrachia tripeira* from Porto MV (Figure 3.3.2, table 3.3.2), has previously been described for *Lamellibrachia* sp. (Naganuma *et al.* 2005) and attributed not only to individual-to-individual (inter-individual) variation but also to part-to-part (intra-individual) variation within a single individual due to a physiologic gradient in the trophosome (de Burgh 1986).

Bacteria belonging to the *Rickettsiales* (*Alphaproteobacteria*) were found in all species except *Lamelisabella denticulata* and *Bobmarleya gadensis*. *Rickettsia* species are obligate endosymbionts of blood-sucking terrestrial arthopods or leeches, but are rare endosymbionts in marine eukaryotes (they have only been identified in a marine ciliate and octocoral) (Weinert *et al*. 2009).

The cluster analysis on the DGGE (Figure 3.3.3) results revealed no patterns between species, depth, mud volcano or age of animal and therefore seems to suggest that they do not seem to select for specific endosymbionts.

Phylogenetic analysis of a few bacterial 16S rRNA gene clones from some of the specimens analyzed in this study confirmed that most of the dominant bacteria identified by PCR_DGGE fall within the *Gammaproteobacteria*. This is in agreement with the results obtained in several frenulata studies showing that many thiotrophic and methanotrophic symbionts of marine invertebrates are *Gammaproteobacteria* (e.g. Kimura *et al*. 2003; Lösekann *et al*. 2008; Thornill *et al*. 2008).

Future studies are essential to understand the role of symbionts in frenulate species, namely to assess their metabolic potential by sequencing target functional genes encoding proteins involved in the sulphide oxidation pathway and carbon fixation. The characterization of the free-living endosymbionts in the sediment of mud volcanoes in the Gulf of Cadiz is also fundamental, since the results may provide clue as to how endosymbionts are acquired in Frenulata species. Such studies are especially important considering the wide global distribution of siboglinids and their potential importance as representatives of the most common symbioses in the deep sea.

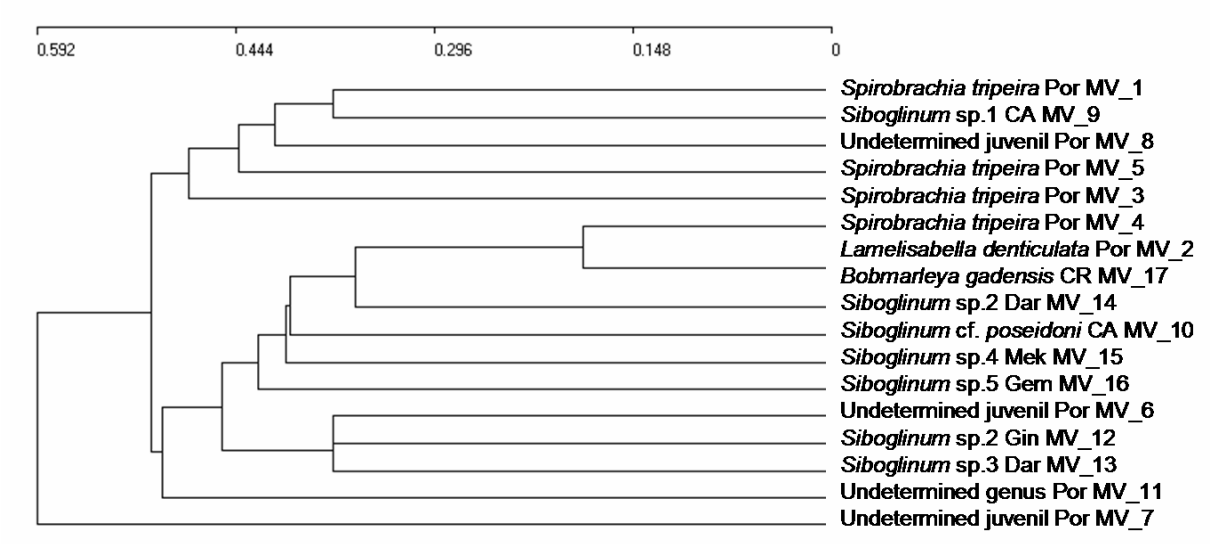

Figure 3.3.3. Cluster analysis of DGGE band pattern shown in figure 3.3.2. Agglomerative cluster analysis was carried out using the software package COMMUNITY ANALYSIS PACKAGE version 3.1 (Pisces Conservation Ltd). Dendograms were constructed by average linkage and average distance method (Webster *et al*. 2007).

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SECTION 4. FINAL REMARKS

Final remarks

In order to deal with the wide environmental heterogeneity of the Gulf of Cadiz, the study of the macrofaunal assemblages was directed towards an extensive sampling of the highest number of different mud volcanoes using a different array and sampling gear and methodologies. As a result of this approach we produced a comprehensive taxonomic list of over 700 taxa from which 507 were identified to species or genus level. This may be considered one of the main achievements of the work carried out in the Gulf of Cadiz as it constitutes a rare exception in cold seep studies. For instance, in one of the most studied cold seep areas in the Western Atlantic, the Gulf of Mexico, only 45 taxa were identified (Cordes *et al*. 2008) and there are no comprehensive species lists of the macrofauna in other well studied areas of the Eastern Atlantic (e.g. the Nordic margin). In fact, one of the major gaps in the present knowledge of cold seep assemblages is the low taxonomic resolution of most published studies particularly in what concerns the accompanying macrofauna.

The assemblages of the Gulf of Cadiz showed high variability in structure, composition and density and therefore the extensive approach used for the study revealed the insufficiency of sampling and replication to fully document the biodiversity and understand the zonation and other distributional patterns at the single mud volcano level. However, there were some important trends that were clearly detected. The benthic assemblages from the mud volcanoes in the shallow and western Moroccan fields show high biodiversity and density, mainly of background non-seep fauna with low degree of endemicity. These features are more pronounced in the shallow Moroccan field where the proximity to the coast as well to the euphotic layer greatly enhances the availability of different food sources and the productivity of the area. The typical seep fauna is represented by a lower number of inconspicuous species such as the small-sized *Siboglinum* spp. and buried bivalves (Solemyidae and Lucinidae). In the deeper mud volcanoes species richness and density of the assemblages are generally much lower but the degree of endemicity is clearly higher and mud volcanoes are colonized by a high diversity of typical seep fauna. Large-sized Siboglinidae sometimes forming conspicuous clumps and

fields of Thyasiridae or Vesicomyidae bivalves may reach high densities in patchy aggregations.

The stabe isotope studies were crucial to show that the species inhabiting the crater of the mud volcanoes derive the bulk of their nutrition from chemotrophic sources although the export of chemotrophic production from mud volcanoes to the adjacent habitats is likely of little relevance. Different species of Frenulata (Siboglinidae) and Bivalvia (Solemyidae, Lucinidae and Thyasiridae) showed a range of isotopic values consistent with either thiotrophic or methanotrophic nutrition. The indication by stable isotope analysis that autotrophic bacteria make a substantial contribution to the nutrition of their hosts, led us to investigate the bacterial symbionts and their phylogenetic relationships based on molecular methods. The results further confirmed the presence of symbionts within gill tissues of bivalves and a great microbial diversity, which include symbionts and other bacterial species with a most likely role in frenulates. Until now most of the bacteria of the different chemosymbiotic species studied belong to the sulfur-oxidizing bacteria (*Gammaproteobacteria*). Other techniques such as *in situ hybridization* and TEM studies are still needed to prove that these bacteria are symbionts and to determine their location inside the host, as they density and the knowledge of target functional genes are crucial to determine their function. The importance of new symbiont phylotypes and their function are mostly unknown and their discovery at cold seeps open new research lines to be explored in the future.

The number of chemosymbiotic species present in the Gulf of Cadiz is remarkably higher than in other cold seeps. Their diversity and distributional patterns appear to be related to the variability in biogeochemical conditions within and between mud volcanoes. In fact, the lack of integration between biological and environmental data is a major gap that hinders our knowledge on the major drivers of biodiversity in the cold seep assemblages of the Gulf of Cadiz. However, a large amount of data was acquired during past and recent cruises. We expect that as these data become available the relationships between natural drivers and biodiversity can be further investigated.

As shown by the results the Gulf of Cadiz is inhabited by faunal assemblages with differing taxonomical and trophic structures that likely

respond to the variability in food sources either chemosynthetically or photosynthetically derived, availability of substrates (such as carbonate concretions or cold-water stony corals) but also possibly to the hydrological regime of the different water masses. In the Gulf of Cadiz the main regions of enhanced turnover in the bivalve assemblage coincide with vertical oceanographic boundaries (Eastern North Atlantic Central Water, Mediterranean Water; North Atlantic Deep Water) and therefore the water mass structure and temperature may be hypothesised as also playing an important role in determining faunal zonation. In the Gulf of Cadiz the complex topographic, geological and hydrological settings produce a considerable environmental heterogeneity that enhances faunal turnover adding to its high regional diversity.

Part of the explanation of the high biodiversity of the area is supported by the geologic history of the central Atlantic that started some 180-160 Ma ago with the formation of the Tethys Seaway. The colonization of the deep Atlantic is hypothesized to have had four major points of entry from which the earliest and most important was Tethyan. According to these authors, the present highest diversity of the Western European basin reflects the fauna's ancient Tethyan origin suplplemented later by othter additions of deep water species. The Gulf of Cadiz (the present day reminiscent of the ancient Tethyan point of entry) is therefore one of the older, if not the oldest deep-sea regions of the Western basin.

The biogeographical context brings the Gulf of Cadiz to the position of a key-site for the interpretation of phylogeographical patterns and evolutionary history of marine organisms along the European margin. Moreover, the Gulf of Cadiz has a strategic central location in relation to other known seeps (Nordic margin, Western Mediterranean, Gulf of Guinea) and vents (Mid-Atlantic Ridge). The knowledge on cold seep communities in this region may be of utmost importance for understanding dispersal of chemosynthetic organisms and connectivity among cold seeps and between cold seep and vent habitats.

ANNEXES

ANNEX I

Table 1: List of samples taken for macrofaunal studies in the Gulf of Cadiz. All sites located in cold seeps (mud volcanoes) and adjacent habitats (cold-water corals and carbonates) are included. Different quantitative (USNEL boxcore, Megacore/Multicore (MC) and NIOZ boxcore, ROV operated pushcores (PUC), colonization devices (TRAC) and lander chambers) and non-quantitative samplers (geological dredge, TV-Grab, ROV operated suction and scoop samples) were used in different cruise.

Location	Cruise-Dive	Station	Date	Latitude	Longitude	Depth	Sampler
Mud volcanoes							
Al Idrisi							
	TTR12	AT412D	2002.07.17	35º14.193'N	06º36.609'W	230	Dredge
Mercator							
	TTR12	AT409D	2002.07.16	35º17.929'N	06º38.903'W	375	Dredge
	TTR12	AT410D	2002.07.16	35º18.014'N	06º38.986'W	392	Dredge
	TTR15	AT569GR	2005.07.25	35º17.917'N	06º38.717'W	358	TV-Grab
	TTR15	AT575B	2005.07.26	35º17.903'N	06º38.715'W	355	USNEL
	TTR15	AT576B	2005.07.26	35º17.657'N	06º39.129'W	428	USNEL
	TTR15	AT577B	2005.07.26	35º17.305'N	06º39.672'W	485	USNEL
	MSM01-03	237-MUC12	2006.05.06	35º17.914'N	06º38.687'W	353	MC
	MSM01-03	241-GKG12	2006.05.06	35º17.918'N	06º38.717'W	353	USNEL
	MSM01-03	242-GKG13	2006.05.06	35º17.870'N	06º38.810'W	350	USNEL
	MSM01-03	267-MUC13	2006.05.09	35º17.875'N	06º38.789'W	350	MC
	MSM01-03	287-MUC14	2006.05.11	35º17.890'N	06º39.059'W	379	MC
	JC10-Isis28	021-CS01	2007.05.19	35º17.916'N	06º38.709'W	354	TRAC
	JC10-Isis28	021-PUC02	2007.05.19	35º17.914'N	06º38.709'W	354	PUC
	JC10-Isis28	021-PUC03	2007.05.19	35º17.914'N	06º38.709'W	354	PUC
	JC10-Isis28	021-PUC04	2007.05.19	35º17.914'N	06º38.709'W	354	PUC
Fíuza							
	TTR14	AT566GR	2004.08.09	35º15.510'N	06º41.702'W	414	TV-Grab
Kidd							
	TTR14	AT528GR	2004.08.03	35º25.304'N	06º43.972'W	489	TV-Grab
	TTR14	AT559B	2004.08.08	35º24.777'N	06º43.782'W	552	USNEL
	TTR14	AT560B	2004.08.08	35º25.306'N	06º43.976'W	498	USNEL
	TTR14	AT561B	2004.08.08	35º25.602'N	06º44.099'W	526	USNEL
Pipoca							
	64PE284	Geob-12705	2008.02.21	36º27.60'N	07º12.33'W	525	NIOZ
	64PE284	Geob-12706	2008.02.21	36º26.81'N	07º12.70'W	702	NIOZ
TTR							
	TTR12	AT415D	2002.07.17	35º21.880'N	06º52.000'W	695	Dredge
	TTR12	AT416GR	2002.07.17	35º21.870'N	06º52.000'W	695	TV-Grab
Meknès							
	TTR14	AT541GR	2004.08.05	34º59.103'N	07º04.435'W	703	TV-Grab
	TTR15	AT581GR	2005.07.28	34º59.178'N	07º04.353'W	700	TV-Grab
	TTR15	AT586GR	2005.07.28	34º59.146'N	07º04.380'W	701	TV-Grab
	MSM01-03	319-GKG21	2006.05.14	34º59.100'N	07º04.439'W	695	USNEL
	MSM01-03	321-GKG22	2006.05.14	34º58.796'N	07º04.394'W	732	USNEL
	MSM01-03	335-GKG23	2006.05.15	34º59.035'N	07º04.552'W	703	USNEL
	64PE284	Geob-12748	2008.03.01	35º58.85'N	07º04.39'W	722	NIOZ
Student							
	TTR10	AT239GR	2000.07.22	35º30.853'N	07º08.816'W	955	TV-Grab
Yuma							
	TTR14	AT524GR	2004.08.02	35º24.973'N	07º05.461'W	960	TV-Grab
	TTR16	AT604GR	2006.05.29	35º25.820'N	07º06.330'W	1030	TV-Grab
	TTR16	AT605GR	2006.05.29	35º25.046'N	07º05.450'W	975	TV-Grab
Ginsburg							
	TTR16	AT607GR	2006.05.29	35º22.677'N	07º04.979'W	983	TV-Grab
Aveiro							
	TTR12	AT395GR	2002.07.11	35º52.226'N	07º26.282'W	1094	TV-Grab
Jesus Baraza							
	TTR12	AT391GR	2002.07.09	35º35.439'N	07º12.264'W	1105	TV-Grab
Darwin							
	TTR16	AT608GR	2006.05.30	35º23.531'N	07º11.475'W	1115	TV-Grab
	JC10-Isis33	032-SUS01	2007.05.21	35º23.523'N	07º11.513'W	1109	Suction
	JC10-Isis33	032-SUS02	2007.05.22	35º23.523'N	07º11.513'W	1110	Suction
	JC10-Isis33	032-CS01	2007.05.21	35º23.523'N	07º11.513'W	1110	TRAC
	JC10-Isis34	036-PUC09	2007.05.22	35º23.541'N	07º11.508'W	1112	PUC

Location Cruise-Dive	Station	Date	Latitude	Longitude	Depth	Sampler
JC10-Isis41	076-SUS1	2007.06.01	35º23.525'N	07º11.511'W	1109	Suction
JC10-Isis41	076-Bibo1	2007.06.01	35º23.503'N	07º11.498'W	1109	Scoop
JC10-Isis41	076-Bibo2	2007.06.01	35º23.540'N	07º11.481'W	1109	Scoop
JC10-Isis41	076-Bibo3	2007.06.01	35º23.533'N	07º11.481'W	1109	Scoop
JC10-Isis41	076-SUS2	2007.06.01	35º23.532'N	07º11.479'W	1109	Suction
TTR17	AT664GR	2008.06.26	35º23.520'N	07º11.485'W	1128	TV-Grab
Chechouan						
TTR16	AT610GR	2006.05.30	35º28.468'N	07º15.477'W	1177	TV-Grab
Captain Arutyunov						
TTR12	AT393GR	2002.07.09	35º39.740'N	07º19.942'W	1327	TV-Grab
TTR12	AT399GR	2002.07.13	35º39.805'N	07º19.997'W	1339	TV-Grab
TTR14	AT546GR	2004.08.06	35º39.692'N	07º20.046'W	1345	TV-Grab
TTR14	AT547GR	2004.08.06	35º39.701'N	07º20.037'W	1344	TV-Grab
MSM01-03	180-GKG07	2006.04.27	35º39.740'N	07º19.960'W	1323	USNEL
MSM01-03	190-MUC08	2006.04.28	35º39.665'N	07º19.970'W	1322	MC
MSM01-03	190-MUC09	2006.04.28	35º39.668'N	07º19.970'W	1320	MC
MSM01-03	194-TVG01	2006.04.28	35º39.282'N	07º20.012'W	1379	TV-Grab
MSM01-03	195-TVG02	2006.04.28	35º39.274'N	07º20.013'W	1390	TV-Grab
MSM01-03	217-GKG10	2006.04.30	35º39.642'N	07º20.049'W	1321	USNEL
MSM01-03	222-MUC10	2006.05.01	35º39.700'N	07º20.011'W	1318	MC
MSM01-03	225-BIGO2	2006.05.04	35º39.707'N	07º20.020'W	1322	Lander
MSM01-03	274-BIGO3	2006.05.10	35º39.738'N	07º20.010'W	1321	Lander
MSM01-03	344-FLUFO5	2006.05.16	35º39.698'N	07º20.006'W	1318	Lander
Sagres						
TTR17	AT667GR	2008.06.27	36º02.199'N	08º05.545'W	1562	TV-Grab
Carlos Ribeiro						
TTR16	AT615GR	2006.05.31	35º47.238'N	08º25.272'W	2200	TV-Grab
MSM01-03	157-GKG05	2006.04.23	35º47.270'N	08º25.360'W	2200	USNEL
MSM01-03	168-MUC06	2006.04.25	35º47.255'N	08º25.354'W	2200	MC
MSM01-03	169-MUC07	2006.04.25	35º47.256'N	08º25.361'W	2199	MC
MSM01-03	184-FLUFO3	2006.04.27	35º47.260'N	08º25.346'W	2200	Lander
JC10	050	2007.05.26	35º47.221'N	08º25.292'W	2176	MC
JC10-Isis36	051-PUC01	2007.05.27	35º47.244'N	08º25.282'W	2197	PUC
JC10-Isis36	051-PUC02	2007.05.27	35º47.244'N	08º25.282'W	2197	PUC
JC10-Isis36	051-PUC03	2007.05.27	35º47.244'N	08º25.282'W	2197	PUC
JC10-Isis36	051-PUC04	2007.05.27	35º47.091'N	08º25.306'W	2240	PUC
JC10-Isis36	051-PUC05	2007.05.27	35º47.091'N	08º25.306'W	2240	PUC
JC10-Isis36	051-PUC06	2007.05.27	35º47.091'N	08º25.306'W	2240	PUC
JC10-Isis36	051-CS01	2007.05.27	35º47.244'N	08º25.282'W	2197	TRAC
JC10	054	2007.05.27	35º47.300'N	08º25.219'W	2179	MC
JC10	057	2007.05.28	35º47.250'N	08º25.324'W	2175	MC
JC10	061	2007.05.29	35º47.073'N	08º25.218'W	2190	MC
JC10	062	2007.05.29	35º47.170'N	08º25.246'W	2180	MC
JC10	063	2007.05.29	35º47.197'N	08º25.269'W	2177	MC
Unnamed mound						
TTR17	AT673GR	2008.06.28	35º30.520'N	08º27.660'W	2368	TV-Grab
Bonjardim						
TTR15	AT597GR	2005.08.02	35º27.563'N	09º00.030'W	3061	TV-Grab
MSM01-03	133-GKG01	2006.0419.	35º27.821'N	09º00.128'W	3049	USNEL
MSM01-03	139-MUC02	2006.04.20	35º27.559'N	08º59.884'W	3054	MC
TTR17	AT676GR	2008.06.29	35º27.527'N	08º59.828'W	3062	TV-Grab
TTR17	AT678GR	2008.06.30	35º27.529'N	08º59.827'W	3060	TV-Grab
Semenovich						
TTR17	AT679GR	2008.06.30	35º13.433'N	09º05.186'W	3265	TV-Grab
Porto						
TTR16	AT622GR	2006.06.03	35º33.773'N	09º30.416'W	3902	TV-Grab
MSM01-03	145-GKG03	2006.04.21	35º33.699'N	09º30.437'W	3860	USNEL
MSM01-03	160-MUC03	2006.04.24	35º33.753'N	09º30.501'W	3863	MC
MSM01-03	161-MUC04	2006.04.24	35º33.754'N	09º30.499'W	3864	MC
MSM01-03	162-MUC05	2006.04.24	35º33.738'N	09º30.492'W	3863	MC
MSM01-03	167-FLUFO2	2006.04.25	35º33.770'N	09º30.520'W	3863	Lander
TTR17	AT683GR	2008.07.01	35º33.750'N	09º30.365'W	3890	TV-Grab
TTR17	AT685GR	2008.07.01	35º33.784'N	09º30.382'W	3870	TV-Grab
TTR17	AT687GR	2008.07.01	35º33.774'N	09º30.429'W	3887	TV-Grab
Carbonate and						

Location Cruise-Dive	Station	Date	Latitude	Longitude	Depth	Sampler
Coral						
W of Gibraltar						
TTR14	AT550D	2004.08.07	35º42.257'N	06º30.000'W	392	Dredge
TTR14	AT551D	2004.08.07	35º42.769'N	06º30.305'W	393	Dredge
TTR14	AT552GR	2004.08.07	35º42.816'N	06º30.234'W	428	TV-Grab
Vernadsky Ridge						
TTR15	AT574D	2005.07.26	35º25.982'N	06º46.661'W	508	Dredge
64PE284	Geob-12759	2008.03.04	35º26.57'N	06º46.78'W	524	NIOZ
Ibérico						
TTR11	AT335D	2001.08.25	36º07.405'N	07º41.198'W	905	Dredge
Guadalquivir Ridge						
TTR11	AT339D	2001.08.26	36º07.591'N	07º46.587'W	1021	Dredge
Formosa Ridge						
TTR12	AT388GR	2002.07.08	36º10.263'N	07º43.819'W	1079	TV-Grab
TTR12	AT389D	2002.07.08	36º10.123'N	07º44.121'W	1068	Dredge
Cadiz Channel						
TTR15	AT599D	2005.08.03	36º06.538'N	07º53.942'W	1275	Dredge
Pen Duick Escarp						
TTR17	AT650GR	2008.06.24	35º16.154'N	06º33.431'W	326	TV-Grab
TTR17	AT652GR	2008.06.25	35º18.913'N	06º37.225'W	399	TV-Grab
TTR17	AT654GR	2008.06.25	35º18.948'N	06º37.275'W	395	TV-Grab
TTR14	AT565GR	2004.08.09	35º18.180'N	06º47.656'W	544	TV-Grab
TTR12	AT406GR	2002.07.15	35º18.148'N	06º47.666'W	550	TV-Grab
TTR12	AT407GR	2002.07.15	35º17.695'N	06º47.082'W	560	TV-Grab
TTR16	AT600GR	2006.05.28	35º18.779'N	06º48.453'W	610	TV-Grab
TTR16	AT602GR	2006.05.28	35º17.693'N	06º47.089'W	556	TV-Grab
Mercator CMP						
64PE284	Geob-12754	2008.03.03	35º20.10'N	06º39.69'W	434	NIOZ
Yuma CMP						
64PE284	Geob-12712	2008.02.23	35º22.27'N	06º54.20'W	733	NIOZ
64PE284	Geob-12721	2008.02.25	35º18.59'N	06º59.94'W	868	NIOZ
64PE284	Geob-12722	2008.02.25	35º18.63'N	07º00.99'W	907	NIOZ
Meknès CMP						
64PE284	Geob-12739	2008.02.28	35º00.01'N	07º04.47'W	736	NIOZ
Central CMP						
64PE284	Geob-12729	2008.02.26	35º10.83'N	06º56.53'W	754	NIOZ

ANNEX II

Table I – Preliminary list of taxa found in the Gulf of Cadiz. Taxonomic data according WoRMS – World Register of Marine Species (http://www.marinespecies.org)

Phyllum PORIFERA Grant, 1836

Class Demospongiae Sollas, 1885

Order Astrophorida Sollas, 1887

Family Ancorinidae Schmidt, 1870

***Jaspis incrustans* (Topsent, 1890)**

Family Ancorinidae Schmidt, 1870

***Poecillastra compressa* (Bowerbank, 1866)**

***Thenea muricata* (Bowerbank, 1858)**

***Vulcanella aberrans* (Maldonado & Uriz, 1996)**

***Pachastrella monilifera* Schmidt, 1868**

Order Dyctyoceratida Minchin, 1900

Family Dysideidae Gray, 1867

Dysidea sp.

Order Halichondrida Gray, 1867

Family Axinellidae Carter, 1875

***Axinella vallerea* Topsent, 1904**

***Phakelia vertilabrum* (Linnaeus, 1767).**

Family Halichondriidae Gray, 1867

***Topsentia glabra* (Topsent, 1898)**

Order Haploscerida Topsent, 1928

Family Chalinidae Gray, 1867

Haliclona aff. *Cratera* (Schmidt, 1862

Family Petrosiidae Van Soest, 1980

Xestospongia sp.

Order Lithistida Schmidt, 1870

Family Theonellidae Lendenfeld, 1903

***Discodermia ramifera* Topsent, 1892**

Order Poecilosclerida Topsent, 1928

Family Cladorhizidae Dendy, 1922

***Asbestopluma* sp**

***Asbestopluma pennatula* (Schmidt, 1875)**

Family Hamacanthidae Gray, 1872

***Hamacantha papillata* Vosmaer, 1885**

Family Hymedesmiidae Topsent, 1928

***Hymedesmia loevistylus* Lundbeck, 1910**

Family Podospongiidae de Laubenfels, 1936

***Podospongia loveni* (Bocage, 1869)**

Classe Hexactinellida Schmidt, 1870

Order Amphidiscosida Schrammen, 1924

Family Hyalonematidae Gray, 1857

Hyalonema sp.

Order Hexactinosida Schrammen, 1912

Family Aphrocallistidae Gray, 1867

Aphrocallistes sp.

Order Lyssacinosida Zittel, 1877

Family Rossellidae Schulze, 1885

Rossella sp.

Phyllum CNIDARIA Hatscheck, 1888

Class Anthozoa Ehrenberg, 1824

Subclass Ceriantipatharia Van Beneden, 1898

Order Anthipatharia Milne-Edwards & Haime, 1857

***Anthipatharia* indeterminado**

Subclass Hexacorallia

Order Actiniaria

Actiniaria indeterminado

SubOrder Thenaria Carlgren, 1899

Family Hormathiidae Carlgren, 1932

Genus *Amphianthus* Hertwig, 1882

Amphianthus sp.

Order Scleractinia Bourne, 1900

Scleractinia indeterminado

SubOrder Caryophylliina Vaughan & Wells, 1943

Family Caryophylliidae Dana, 1846

Genus *Caryophyllia* Lamark, 1801

***Caryophyllia* sp.**

Genus *Desmophyllum* Ehrenberg, 1834

***Desmophyllum cristagalli* Milne-Edwards & Haime, 1848**

Genus *Lophelia* Milne-Edwards & Haime, 1849

***Lophelia pertusa* (Linnaeus, 1758)**

Family Guyniidae Hickson, 1910

Genus *Stenocyathus* De Pourtalès, 1871

cf. *Stenocyathus* cf. *vermiformis* (De Pourtalès, 1868)

SubOrder Faviina Vaughan & Wells, 1943

Family Oculinidae Gray, 1847

Genus *Madrepora* Linnaeus, 1758

***Madrepora oculata* Linnaeus, 1758**

Order Zoanthidea

Family Epizoanthidae Gray, 1867

Genus *Epizoanthus* Gray, 1867

***Epizoanthus* sp.**

Family Parazoanthidae

Genus *Parazoanthus* Haddon & Shackleton, 1891

***Parazoanthus* sp.**

Subclass Octocorallia Haeckel, 1866

Order Alcyonacea Lamouroux, 1865

Family Clavulariidae Hickson, 1894

Genus *Clavularia* de Blainville, 1830

***Clavularia* sp1**

***Clavularia* sp2**

Genus *Telestula* Madsen, 1914

cf. *Telestula* sp.

Família Dendrobrachiidae Brook, 1889

Genus *Dendrobrachia* Brook, 1889

***Dendrobrachia* sp.**

SubOrder Alcyoniina

Family Alcyoniidae Lamouroux, 1812

Genus *Anthomastus* Verril, 1878

***Anthomastus grandiflorus* Verril, 1878**

SubOrder Calcaxonia

Family Elisellidae Gray, 1859

Genus *Nicella* Gray, 1870

***Nicella granifera* (Kölliker, 1865)**

Genus *Viminella* Gray, 1870

***Viminella* cf. *flagellum* (Johnson, 1863)**

Family Isididae Lamouroux, 1812

Genus *Chelidonisis* Studer, 1890

***Chelidonisis aurantiaca* Studer, 1890**

Genus *Isidella* Gray, 1857

***Isidella elongata* (Esper, 1788)**

Family Primnoidae

Genus *Callogorgia* Gray, 1858

***Callogorgia verticilata* (Pallas, 1766)**

SubOrder Holaxonia Studer, 1887

Family Acanthogorgiidae Gray, 1859

Genus *Acanthogorgia* Gray, 1857

***Acanthogorgia armata* Verrill, 1878**

Family Plexauridae Gray, 1859

Genus *Muriceides* Studer, 1887

***Muriceides paucituberculata* (Marion, 1882)**

Genus *Muriceopsis* Aurivillius, 1931

***Muriceopsis* sp1**

***Muriceopsis* sp2**

Genus *Paramuricea* Kölliker, 1865

***Paramuricea* sp.**

Genus *Swiftia* Duchassaing & Michelotti, 1864

***Swiftia dubia* (Thomson, 1929)**

SubOrder Scleraxonia Studer, 1887

Scleraxonia indeterminado

Class Hydrozoa Owen, 1834

SubClass Hydroidolina

Order Anthoathecatae Cornellius, 1992
SubOrder Filifera Kühn, 1913
Family Tubiclavoididae Moura, Cunha & Schuchert, 2007
Genus *Tubiclavoides* Moura, Cunha & Schuchert, 2007
***Tubiclavoides striatum* Moura, Cunha & Schuchert, 2007**
Family Eudendriidae L. Agassiz, 1862
Genus *Eudendrium* Ehrenberg, 1834
Eudendrium rameum (Pallas, 1766)
***Eudendrium* sp1**
***Eudendrium* sp2**
***Eudendrium* sp3**
***Eudendrium* sp4**
***Eudendrium* sp5**
***Eudendrium* sp6**
***Eudendrium* sp7**
Order Leptothecatae
Family Aglaopheniidae Marktanner-Turneretscher, 1890
Genus *Aglaophenia* Lamouroux, 1812
Aglaophenia lophocarpa Allman, 1877
Aglaophenia tubulifera (Hincks, 1861)
Genus *Streptocaulus* Allman, 1883
Streptocaulus* cf. *corneliusi Ramil & Vervoort, 1992
Streptocaulus dollfusi (Billard, 1924)
Genus *Lytocarpia* Kirchenpauer, 1872
Lytocarpia myriophyllum (Linnaeus, 1758)
Family Campanulariidae 1836
Campanulariidae indeterminado
Genus *Campanularia* Lamarck, 1816
Campanularia hincksii Alder, 1856
Genus *Clytia* Lamouroux, 1812
***Clytia* sp.**
Clytia hemisphaerica (Linnaeus, 1767)
Clytia* cf. *hemisphaerica (Linnaeus, 1767)
Clytia linearis (Thornely, 1899)
Genus *Laomeda* Lamouroux, 1812
cf. *Laomedea neglecta* Alder, 1856
Genus *Obelia* Péron & Lesueur, 1810
Obelia* cf. *dichotoma (Linnaeus, 1758)
Obelia geniculata (Linnaeus, 1758)
Family Campanulinidae Hincks, 1868
Campanulinidae indeterminado
Genus *Campanulina* van Beneden, 1847
Campanulina paniculata Sars, 1873
cf. *Campanulina paniculata* 1 Sars, 1873
cf. *Campanulina paniculata* 2 Sars, 1873

Genus *Cuspidella* Hincks, 1866

***Cuspidella* sp.**

Genus *Lafoeina* G.O. Sars, 1874

Lafoeina tenuis G.O. Sars, 1874

Family Haleciidae Hincks, 1868

Genus *Halecium* Oken, 1815

***Halecium* sp1**

***Halecium* sp2**

Halecium sessile Norman, 1867

Halecium sibogae marocanum Billard, 1934

Halecium tenellum Hincks, 1861

Family Halopteridae Millard, 1962

Genus *Antennella* Allman, 1877

Antennella secundaria (Gmelin, 1791)

Family Kirchenpaueriidae Millard, 1962

Genus *Kirchenpaueria* Jickeli, 1883

Kirchenpaueria bonnevieae (Billard, 1906)

Kirchenpaueria pinnata (Linnaeus, 1758)

Family Lafoeidae A. Agassiz, 1865

SubFamily Lafoeinae A. Agassiz, 1865

Genus *Acryptolaria* Norman, 1875

Acryptolaria conferta (Allman, 1877)

Acryptolaria longitheca (Allman, 1877)

Genus *Cryptolaria* Busk, 1857

Cryptolaria pectinata (Allman, 1888)

Genus *Filellum* Hincks, 1868

Filellum* cf. *serratum (Clarke, 1879)

Genus *Lafoea* Lamouroux, 1821

Lafoea dumosa (Fleming, 1820)

SubFamily Zygophylacinae Quelch, 1885

Genus *Zygophylax* Quelch, 1885

Zygophylax bathyphila Leloup, 1940

Zygophylax biarmata Billard, 1905

Zygophylax levinseni (Saemundsson, 1911)

Family Lovenellidae Russel, 1953

Genus *Lovenella* Hincks, 1868

Lovenella producta (G.O. Sars, 1874)

Family Plumulariidae McCrady, 1859

***Plumulariidae* indeterminado**

***Plumulariinae* indeterminado**

Genus *Nemertesia* Lamouroux, 1812

***Nemertesia* sp (2 species)**

Nemertesia antennina (Linnaeus, 1758)

Nemertesia ventriculiformis (Marktanner, 1890)

Genus *Polyplumaria* Sars, 1873

Polyplumaria flabellata Sars, 1873

Family Sertulariidae Lamouroux, 1812

Genus *Diphasia* L. Agassiz, 1862

Diphasia margareta (Hassal, 1841)

Diphasia pinastrum (Cuvier, 1830)

Genus *Salacia* Lamouroux, 1816

Salacia desmoides (Torrey, 1902)

Genus *Sertularella* Gray, 1848

Sertularella gayi gayi (Lamouroux, 1821)

Sertularella gayi robusta Allman, 1873

Sertularella polyzonias (Linnaeus, 1758)

Family Tiarannidae Russel, 1940

Genus *Modeeria* Forbes, 1848

Modeeria rotunda (Quoy & Gaimard, 1827)

SubPhyllum Medusozoa

Class Scyphozoa Götte, 1887

Order Coronatae

Family Nausithoidae

Genus *Nausithoe* Kölliker, 1853

***Nausithoe* sp.**

Phyllum NEMERTINA

Undetermined

Phyllum SIPUNCULA

Undetermined

Phyllum ECHIURA

Undetermined

hyllum CEPHALORHYNCHA

Class Priapulida

Undetermined

Phyllum ANNELIDA

Class Clitellata

… Subclass Oligochaeta

Undetermined

Class Polychaeta Grube 1850

Subclass Scolecida

Order Capitellida

Family Capitellidae Grube 1862

Undetermined

Genus *Notomastus* Sars 1850

***Notomastus* sp.**

Genus *Paracapitella*

***Paracapitella* sp.**

Family Maldanidae Malmgren 1867

Undetermined

Genus *Euclymene* Verrill 1900

Euclymene* cf *oerstedi (Claparède 1863)

Genus *Praxillella* Verrill 1881

***Praxillella* sp**.

Order Cossurida

Family Cossuridae Day 1963

Undetermined

Order Opheliida

Family Ophelidae

Undetermined

Genus *Ophelina* Örsted 1843

Cf. Ophelina cylindricaudata (Hansen 1878)

Family Scalibregmidae Malmgren 1867

Undetermined

Order Orbiniida

Family Orbiniidae Hartman 1942

Genus *Leitoscoloplos* Day 1977

Leitoscoloplos cf. mammosus Mackie 1987

Genus *Orbinia* Quatrefages 1865

Orbinia sp.

Family Paraonidae Cerruti 1909

Undetermined

Genus *Aricidea* Webster 1879

Aricidea abyssalis Laubier & Ramos 1974

Aricidea annae Laubier 1967

Aricidea cerruti Laubier 1966

Aricidea fragilis mediterranea Laubier & Ramos 1974

Aricidea simonae Laubier & Ramos 1974

Aricidea suecica meridionalis Laubier & Ramos 1974

Genus *Cirrophorus* Ehlers 1908

Cirrophorus lyriformis (Annenkova 1934)

Genus *Levinsenia* Mesnil 1897

Levinsenia gracilis (Tauber 1879)

Genus *Paraonides* Cerruti 1909

Paraonides lyra Griffiths 1976

Subclass Palpata

(Aciculata)

Order Amphinomida

Family Amphinomidae Savigny *in* Lamarck 1818

Genus *Pareurythoe* Gustafson 1930

Pareurythoe borealis Gustafson 1930

Family Euphrosinidae Williams 1851

Genus *Euphrosine* Lamarck 1818

Euphrosine armadillo Sars 1851

Order Eunicida
- Family Dorvilleidae Chamberlin 1919
  - Genus *Dorvillea* Parfitt 1866
    - ***Dorvillea* sp.**
- Family Eunicidae Savigny 1818
  - Genus *Eunice* Cuvier 1817
    - ***Eunice dubitatus*** Fauchald 1974
    - ***Eunice norvegica*** (Linné 1767)
    - ***Eunice vittata*** (Delle Chiaje 1828)
  - Genus *Lysidice* Lamarck 1818
    - ***Lysidice ninetta*** Audouin & Milne-Edwards 1833
  - Genus *Marphysa* Quatrefages 1865
    - ***Marphysa belli*** Audouin & Milne-Edwards 1833
  - Genus *Nematonereis* Schmarda 1861
    - ***Nematonereis unicornis*** Schmarda 1861
- Family Lumbrineridae Malmgren 1867
    - **Undetermined**
  - Genus *Lumbrinerides* Orensanz 1973
    - ***Lumbrinerides laubieri*** Miura 1980
  - Genus *Lumbrineriopsis* Orensanz 1973
    - ***Lumbrineriopsis paradoxa*** (Saint Joseph 1888)
  - Genus *Lumbrineris* Blainville 1828
    - ***Lumbrineris* sp.**
    - ***Lumbrineris emandibulata*** Ramos, 1976
    - ***Lumbrineris fragilis*** (O. F. Müller 1776)
    - ***Lumbrineris gracili****s* (Ehlers 1868)
    - ***Lumbrineris latreilli*** Audouin & Milne-Edwards 1834
  - Genus *Ninoe* Kinberg 1864
    - ***Ninoe* sp.**
- Family Oenonidae
  - Genus *Drilonereis* Claparède 1870
    - ***Drilonereis filum*** (Claparede 1870)
- Family Onuphidae Kinberg 1865
    - **Undetermined**
  - Genus *Hyalinoecia* Malmgren 1867
    - ***Hyalinoecia tubicola*** (O. F. Mueller 1788)
  - Genus *Paradiopatra* Ehlers 1887
    - ***Paradiopatra hispanica*** Amoureux 1972

Order Phyllodocida
- Family Acoetidae Kinberg 1856
  - Genus *Panthalis* Kinberg 1856
    - ***Panthalis cf. oerstedi*** Kinberg 1856
- Family Chrysopetalidae Ehlers 1864
  - Genus *Arichlidon* Watson Russell 1998
    - ***Arichlidon reyssi*** (Katzmann, Laubier & Ramos 1974)
- Family Glyceridae Grube 1850

Genus *Glycera* Savigny 1818

Glycera lapidum Quatrefages 1865

Glycera rouxi Audouin & Milne-Edwards 1833

Glycera tesselata Grube 1863

Genus *Glycerella* Arwidsson 1899

Glycerella atlantica Wesenberg-Lund 1950

Family Goniadidae Kinberg 1866

Undetermined

Family Hesionidae Sars 1862

Genus *Leocatres* Kinberg 1866

Leocrates atlanticus (McIntosh 1885)

Family Nephtyidae

Genus *Nephtys* Cuvier 1817

***Nephtys* sp.**

Nephtys hystricis McIntosh 1900

Nephtys paradoxa Macquart 1846

Family Nereididae Johnston 1865

Undetermined

Genus *Eunereis* Malmgren 1865

Eunereis longissima Johnston 1840

Genus *Neanthes*

***Neanthes* sp.**

Neanthes cf. irrorata (Malmgren 1867)

Genus *Nicon*

Nicon* cf. *sinica Wu & Sun 1979

Genus *Websterinereis* Pettibone 1971

Websterinereis glauca (Claparede 1870)

Family Pholoidae Kinberg 1857

Genus *Pholoides* Pruvot 1895

Pholoides dorsipapillatus (Marenzeller 1893)

Family Phyllodocidae Williams 1851

Undetermined

Genus *Anaitides* Czerniavsky 1882

Anaitides maculata (Linnaeus 1767)

Anaitides madeirensis Langerhans 1880

Genus *Eulalia* Savigny 1818

***Eulalia* sp**.

Eulalia mustella Pleijel 1987

Genus *Notophyllum* Örsted 1843

Notophyllum foliosum Strobl 1898

Family Pilargidae Saint-Joseph 1899

Genus *Loandalia* Monro 1936

***Loandalia* sp.**

Genus *Otopsis*

***Otopsis* sp.**

Genus *Sigambra*

Sigambra cf. parva (Day 1963)

Genus *Synelmis* Chamberlin 1919

***Synelmis* sp.**

Family Polynoidae Kinberg 1856

Undetermined

Genus *Acholoe* Claparede 1870

Cf. *Acholoe* sp.

Genus *Harmothoe* Kinberg 1856

Harmothoe evei Kirkegaard 1980

Genus *Subadyte* Pettibone 1969

Subadyte pellucida (Ehlers 1864)

Family Sigalionidae Kinberg 1856

Undetermined

Genus *Sigalion* Audouin & Milne-Edwards 1832

***Sigalion* sp.**

Genus *Sthenelais* Kinberg 1856

Sthenelais boa (Johnston 1839)

Sthenelais zetlandica McIntosh 1876

Family Syllidae Grube 1850

Undetermined

Genus *Exogone* Örsted 1845

Exogone sorbei San Martín, Ceberio & Aguirrezabalaga 1996

Exogone cf. verugera (Claparede 1868)

Genus *Haplosyllis* Langerhans 1879

Haplosyllis spongicola (Grube 1855)

Genus *Syllis* Lamarck 1818

Syllis caeca (Katzmann 1973).

Syllis hyalina Grube 1863

Incertae sedis

Family Nautiliniellidae

Genus Natsushima

Natsushima bifurcata Miura & Laubier 1990

Family Sphaerodoridae

Undetermined

(Canalipalpata)

Order Fauveliopsida

Family Fauvelopsidae

Undetermined

Genus *Lauberiopsis* Petersen 2000

Lauberiopsis cabiochi (Amoureux 1982)

Order Flabelligerida

Family Flabelligeridae

Genus *Diplocirrus* Haase 1915

***Diplocirrus* sp.**

Genus *Therochaeta*

***Therochaeta* sp.**

Order Oweniida

Family Oweniidae

Genus *Galathowenia* Kirkegaard 1959

***Galathowenia* sp**

Genus *Myriochele* Malmgren 1867

***Myriochele* sp.**

Order Sabellida

Family Sabellidae

Undetermined

Genus *Ampliglena* Claparede 1864

***Ampliglena* sp.**

Genus *Sabella* Linnaeus 1767

***Sabella* sp.**

Family Serpulidae Rafinesque 1815

Undetermined

Genus *Omphalopomopsis* Saint-Joseph 1894

Omphalopomopsis fimbriata (Delle Chiaje 1828)

Genus *Serpula* Linnaeus 1758

Serpula vermicularis Linnaeus 1767

Genus *Vermiliopsis* Linnaeus 1758

Vermiliopsis infundibulum (Philippi 1844)

Family Siboglinidae

Genus *Bobmarleya* Hilário & Cunha 2008

Bobmarleya gadensis Hilário & Cunha 2008

Genus *Cyclobrachia* Ivanov 1960

Cf. *Cyclobrachia* sp.

Genus *Lamelisabella* Uschakow 1933

***Lamelisabella* sp.**

Lamelisabella denticulata Southward 1978

Genus *Polybrachia* Ivanov 1952

***Polybrachia* spp** (7 species)

Genus *Siboglinum* Caullery 1914

"*Siboglinum*" spp (6 species)

Siboglinum poseidoni Flugel & Langhof 1983

Genus *Spirobrachia* Ivanov 1952

Spirobrachia tripeira Hilário & Cunha 2008

Order Spionida

Family Chaetopteridae

Undetermined

Genus *Chaetopterus* Cuvier 1827

Chaetopterus* cf. *variopedatus (Renier 1804)

Genus *Spiochaetopterus* Sars 1853

***Spiochaetopterus* sp.** (2 species)

Family Cirratulidae Ryckholt 1851

Undetermined

Genus *Aphelochaeta* Blake 1991

***Aphelochaeta* sp.**

Genus *Dodecaceria* Örsted 1843

***Dodecaceria* sp.**

Family Magelonidae Cunningham & Ramage 1888

Genus *Magelona* F. Müller 1858

Magelona cf. minuta Melander 1913

Family Poecilochaetidae

Genus *Poecilochaetus* Claparède 1875

Poecilochaetus* cf. *fulgonis Zlobin 1983

Family Spionidae G.O. Sars 1872

Undetermined

Genus *Laonice* Malmgren 1867

***Laonice* sp.**

Laonice cirrata (Sars 1850)

Genus *Prionospio* Malmgren 1867

***Prionospio* sp**. (4 species)

Prionospio* cf. *aluta Maciolek 1985

Prionospio ehlersi Fauvel 1928

Genus *Scolecolepides* Ehlers 1907

***Scolecolepides* sp. nov**

Genus *Scolelepis* Blainville 1828

***Scolelepis* spp** (3 species)

Genus *Spiophanes* Grube 1860

***Spiophanes* sp.** (1 species)

Spiophanes abyssalis Maciolek 2000

Spiophanes kroyeri Grube 1860

Order Terebellida

Family Ampharetidae Malmgren 1866

Undetermined

Genus *Amphicteis* Grube 1850

Amphicteis midas (Gosse 1855)

Genus *Eclysippe* Eliason 1955

Eclysippe vanelli (Fauvel 1936)

Genus *Lysippides* Hessle 1917

Lysippides* cf. *fragilis Wollenbaek 1912

Genus *Melinnopsis* McIntosh 1885

***Melinnopsis* sp.**

Family Pectinariidae

Genus *Petta* Malmgren 1866

Petta pusilla Malmgren 1866

Family Sabellariidae

Genus *Phalacrostemma* Marenzeller 1895

Phalacrostemma sp.

Genus *Tetreres*

***Tetreres* sp.**

Family Terebellidae Mamlgren 1865

Undetermined

Genus *Amphitrite* O.F. Müller 1771

Amphitrite cirrata (O. F. Müller 1771 in 1776)

Genus *Euthelepus* McIntosh 1885

Euthelepus setubalensis McIntosh 1885

Genus *Lanice* Malmgren 1866

***Lanice* sp.**

Genus *Neoamphitrite* Hessle 1917

***Neoamphitrite* sp.**

Neoamphitrite affinis (Malmgren 1866)

Genus *Polycirrus* Grube 1850

***Polycirrus* sp.**

Family Trichobranchidae Malmgren 1865

Genus *Terebellides* Sars 1835

Terebellides cf. stroemi Sars 1835

Phyllum MOLLUSCA

Class Solenogastres Gegenbaur 1878

Undetermined

Class Polyplacophora Gray 1821

Undetermined

Class Gastropoda Cuvier 1795

Undetermined

SubClass Caenogastropoda Cox 1960

Order Neogastropoda

Family cf. Conidae Rafinesque 1815

Undetermined

Family cf. Drilliidae Olsson 1964

Undetermined

Family cf. Muricidae

Undetermined

Family cf. Turridae

Undetermined

Incertae sedis

Family Cerithiopsidae H. & A. Adams, 1854

Undetermined

Family cf. Eulimidae

Undetermined

Family cf. Triphoridae Gray, 1847

Undetermined

SubClass Heterobranchia

Order Nudibranchia

Undetermined

Incertae sedis

Family Architectonicidae Gray 1850

Undetermined

Family cf. Pyramidellidae Gray 1840

Undetermined

SubClass Prosobranchia Milne-Edwards 1848

Order Mesogastropoda Thiele 1925

Family cf. Rissoidae Gray 1847

Undetermined

Family cf. Triviidae Trochel 1863

Undetermined

Family cf. Tornidae

Undetermined

SubClass Vetigastropoda Salvini-Plawen & Haszprunar 1987

Family cf. Scissurellidae Gray 1847

Undetermined

Family cf. Solariellidae Powell 1951

Undetermined

Family cf. Trochidae Rafinesque 1815

Undetermined

Class Bivalvia Linnaeus, 1758

Order Anomalodesmata

Family Cuspidariidae Dall, 1886

Genus *Cuspidaria* Nardo, 1840

***Cuspidaria* sp**. (4 species)

Cuspidaria inflata (Jeffreys 1882)

Genus *Tropidomya* Dall & Smith, 1886

Tropidomya abbreviata (Forbes, 1843)

Family Poromyidae

Genus *Poromya* Forbes, 1844

Poromya sp.

SubClass Heterodonta Neumayr, 1884

Incertae sedis

Family Hiatellidae Gray, 1824

Genus *Hiatella* Bosc, 1801

Hiatella arctica (Linnaeus 1767)

Family Lucinidae Fleming, 1828

Genus *Lucinoma* Dall, 1901

***Lucinoma* sp. nov**

Family Kelliellidae Fischer, 1887

Genus *Kelliella* M. Sars, 1870

Kelliella abyssicola (Forbes, 1844)

Family Semelidae Stoliczka, 1870

Genus *Abra* Leach in Lamarck, 1818

Abra longicallus (Scacchi, 1834)

Family Thyasiridae Dall, 1901

Genus *Axinulus* Verril & Bush, 1898

Axinulus croulinensis (Jeffreys 1847)

Genus *Leptaxinus* Verril & Bush, 1898

Leptaxinus minutus (Verril & Bush, 1898)

Genus *Mendicula* Iredale, 1924

Mendicula ferruginosa (Forbes 1844)

Genus *Spinaxinus* Oliver & Holmes 2006

Spinaxinus sentosus Oliver & Holmes 2006

Genus *Thyasira* Leach in Lamarck, 1818

Thyasira granulosa (Monterosato 1874)

Thyasira obsoleta (Verrill & Bush 1898)

Thyasira tortuosa (Jeffreys 1881)

Thyasira vulcolutre Rodrigues & Oliver 2008

Family Trapezidae

Genus *Isorropodon* Sturany, 1896

***Isorropodon* sp. nov**

Isorropodon cf. perplexum (Sturany, 1896)

Family Vesicomyidae

Genus *Calyptogena* Dall, 1891

***Calyptogena* sp**.

Genus Vesicomya Dall, 1886

Vesicomya atlantica (Smith 1885)

SubClass Protobranchia

Order Nuculanoida

Family Malletidae H. & A. Adams, 1858

Genus *Malletia* Des Moulins, 1832

Malletia johnsoni Clarke 1961

Family Neilonellidae

Genus *Pseudoneilonella* Laghi, 1986

Pseudoneilonella latior (Jeffreys, 1876)

Family Nuculanidae Meek, 1864

Genus *Ledella* Verrill & Bush, 1897

Ledella messanensis (Jeffreys 1870)

Genus *Nuculana* (Link 1807)

Nuculana commutata (Philippi 1844)

Family Yoldiidae Habe 1977

Genus *Microgloma* Sanders & Allen 1973

***Microgloma* sp.**

Microgloma pusilla (Jeffreys 1879)

Microgloma tumidula (Monterosato 1880)

Genus *Yoldiella* A. E. Verrill & Bush 1897

Yoldiella inconspicua sensu Allen 2001

Order Nuculoida

Family Nuculidae Gray, 1824

Genus *Ennucula* Iredale, 1931

Ennucula aegeensis (Forbes 1844)

Ennucula bushae (Dollfus 1898)

Genus *Nucula Lamarck, 1799*

Nucula sulcata Bronn 1831

Genus *Nuculoma* Cossman 1907

Nuculoma sp.

Family Pristiglomidae

Genus *Pristigloma* Dall, 1900

Pristigloma nitens (Jeffreys, 1876)

Order Solemyoida

Family Solemyidae

Genus *Acharax* Dall 1908

Acharax sp. nov

Genus *Petrasma*

Petrasma sp. nov

SubClass Pteriomorpha

Order Arcoida Stoliczka 1871

Family Arcidae Lamarck 1809

Genus *Bathyarca* Kobelt, 1891

Bathyarca phyllippiana (Nyst 1848)

Genus *Bentharca* Verrill & Bush, 1898

Bentharca asperula (Dall 1881)

Family Limopsidae

Genus *Limopsis* Sassi 1827

Limopsis minuta (Philippi 1836)

Order Mytiloida Ferussac 1822

Family Mytilidae Rafinesque 1815

Genus *Bathymodiolus* Kenk & Wilson, 1985

Bathymodiolus mauritanicus Cosel 2002

Genus *Dacrydium* Torell, 1859

Dacrydium sp.

Dacrydium balgimi Salas & Gofas 1997

Dacrydium wareni Salas & Gofas 1997

Genus *Idas*

***Idas* sp.**

Incertae sedis

Family Anomiidae Rafinesque, 1815

Genus *Heteranomia* Winckworth, 1922

Heteranomia squamula (Linnaeus 1758)

Family Limidae Rafinesque, 1815

Genus *Acesta* Strelzov, 1973

Acesta excavata (Fabricius 1779)

Family Pectinidae Rafinesque, 1815

Genus *Delectopecten* Stewart, 1930

Delectopecten vitreus (Gmelin 1791)

Family Propeamussiidae R.T. Abbott, 1954

Genus *Cyclopecten* A. E. Verrill, 1897

Cyclopecten hoskynsi (Forbes 1844)

Genus *Propeamussium* de Gregorio, 1884

Propeamussium sp.

Family Spondylidae

Genus *Spondylus*

Spondylus gussoni Costa O.G. 1829

Class Scaphopoda Bronn, 1862

Undetermined

Phyllum ARTHROPODA

Class Pycnogonida Latreille 1810

Order Pantopoda Gerstäcker 1863

Undetermined

Family Colossendeidae Jarzynsky 1870

Class Maxillopoda Dahl 1956

InfraClass Cirripedia Burmeister 1834

Order Sessilia Lamarck 1818

Family Verrucidae Darwin, 1854

Genus *Verruca* Schumacher, 1817

***Verruca* sp**.

Class Malacostraca

Superorder Eucarida Calman, 1904

Order Decapoda Latreille, 1803

Infraorder Caridea Dana, 1852

Family Alpheidae Rafinesque, 1815

Genus *Alpheus* Weber, 1795

***Alpheus* sp.**

Family Crangronidae

Genus *Philocheras* Stebbing, 1900

Philocheras* cf. *monachanthus (Holthuis 1961)

Genus *Pontocaris* Bate 1888

Pontocaris lacazei (Gourret 1887)

Family Stylodactylidae Bate 1888

Genus *Stylodactylus*

Stylodactylus serratus Milne-Edwars 1881

Family Callianassidae Dana 1852

Genus *Vulcanocalliax* Dworschak & Cunha 2007

Vulcanocalliax srutyunovi Dworshack & Cunha 2007

Family Galatheidae Samoelle 1819

Genus *Munida Leach 1820*

Munida tenuimana Sars 1872

Genus *Munidopsis* Whiteaves 1874

Munidopsis acutispina Benedict 1902

Family Paguridae Latreille 1802

Genus *Pagurus* Fabricius 1775

Pagurus sp.

Infraorder Brachyura Linnaeus 1758

Family Cymonomidae Bouvier 1897

Genus *Cymonomus* Milne-Edwards 1881

Cymonomus granulatus (Thomson 1973)

Family Goneplacidae MacLeay 1838

Genus *Goneplax* Leach 1814

Goneplax rhomboides (Linnaeus 1758)

Family Homolidae de Haan 1839

Genus *Homola* Leach 1815

Homola barbata (Fabricius 1793)

Family Leucosiidae Samouelle 1819

Genus *Ebalia* Leach 1817

Ebalia granulosa H. Milne-Edwards 1837

Ebalia nux A. Milne-Edwards 1883

Ebalia cf. tuberosa (Pennant 1777)

Family Pilumnidae Samouelle 1819

Genus *Pilumnus* Leach 1815

Pilumnus inermis A Milne-Edwards & Bouvier 1984

Family Portunidae Rafinesque 1815

Genus *Bathynectes* Stimpson 1871

Bathynectes maravigna (Prestandrea 1839)

Family Stenopodidae Claus 1872

Genus *Richardina* Milne-Edwards 1881

Richardina cf. spinicincta A. Milne-Edwards 1881

Family Xanthidae MacLeay 1815

Genus *Monodaeus* Guinot 1967

Monodaeus couchi (Couch 1851)

Order Euphausiacea Dana, 1852

Family Euphausiidae Dana 1852

Genus *Meganyctiphanes* Holt & Tattersall 1905

Meganyctiphanes norvegica (M. Sars 1857)

Superorder Leptostraca

Order Nebaliacea

Family Nebaliidae Samouelle 1819

Genus *Nebali*a Leach 1814

***Nebalia* sp.**

Superorder Peracarida Calman 1904

Order Amphipoda Latreille 1816

Suborder Corophiidea

Family Aoridae Walker 1908

Undetermined

Genus *Lembos* Bate 1857

***Lembos* sp.**

Family Caprellidae Leach 1814

Undetermined

Genus *Caprella* Guilding 1824

Caprella sp.

Caprella septentrionalis Krøyer, 1838

Genus *Liropus* Mayer 1890

Liropus elongatus Mayer 1890

Genus *Parvipalpus* Mayer 1890

Parvipalpus linea Mayer 1980

Genus *Phtisica* Slabber 1778

Phtisica marina Slabber 1769

Genus *Pseudoprotella* Mayer 1890

Pseudoprotella phasma (Montagu 1804)

Family Dulichiidae Dana 1849

Genus *Dulichiopsis* Laubitz 1977

Dulichiopsis nordlandicus (Boeck 1871)

Family Ischyroceridae Stebbing 1899

Undetermined

Genus Jassa Leach 1814

Jassa sp.

Genus *Notopoma*

Notopoma sp. nov

Family Photidae Boeck 1871

Genus *Gammaropsis* Liljeborg 1855

***Gammaropsis* sp.**

Gammaropsis crenulata Krapp-Schickel & Myers 1979

Gammaropsis* cf. *dentata Chevreux 1900

Genus *Photis* Krøyer 1842

Photis longicaudata (Battle & Westwood, 1862)

Family Podoceridae Leach 1814

Genus *Laetmatophilus* Bruzelius 1859

Laetmatophilus ledoyeri Ruffo 1986

Suborder Gammaridea Latreille 1802

Family Ampeliscidae Costa 1857

Undetermined

Genus *Ampelisca* Krøyer 1842

Ampelisca* cf. *anophthalma Bellan-Santini & Kaim-Malka 1977

Ampelisca brevicornis (Costa 1853)

Ampelisca cf. dalmatina Karaman 1975

Ampelisca tenuicornis Liljeborg 1855

Genus *Byblis* Boeck 1871

Byblis* cf. *guernei Chevreux, 1888

Family Amphilochidae Boeck 1871

Genus *Amphilochoides* Sars 1892

Amphilocoides longimanus (Chevreux 1888)

Amphilocoides serratipes (Norman 1869)

Genus *Amphilochus* Bate 1862

***Amphilochus* sp.**

Amphilochus manudens Bate 1862

Family Aristiidae Lowry & Stoddart 1997

Genus *Aristias* Boeck 1871

Aristias cf. neglectus Hansen 1887

Family Atylidae G.O. Sars 1882

Genus *Atylus* Leach 1815

***Atylus* sp**.

Family Carangoliopsidae Bousfield 1977

Genus *Carangoliopsis*

Carangoliopsis spinulosa Ledoyer 1970

Family Colomastigidae Stebbing 1899

Genus *Colomastix* Grube 1861

Colomastix* cf. *pusilla Grube 1861

Family Cressidae Stebbing 1899

Genus Cressa

Cressa cristata Myers 1969

Family Eusiridae Stebbing 1888

Genus *Eusirus* Krøyer 1845

Eusirus longipes Boeck 1861

Genus *Rhachotropis* Boeck 1871

Rhachotropis sp.

Family Iphimediidae Boeck 1871

Genus *Iphimedia* Rathke 1843

***Imphimedia* sp.**

Family Leucothoidae Dana 1852

Genus *Leucothoe* Leach 1814

Leucothoe incisa Robertson 1892

Leucothoe cf. spinicarpa (Abildgaard 1789)

Family Liljeborgiidae Stebbing 1899

Genus *Idunella* Sars 1894

***Idunella* sp.**

Genus *Liljeborgia* Bate 1862

***Liljeborgia* sp**.

Family Lysianassidae Dana 1849

Undetermined

Genus *Hippomedon* Boeck 1871

Hippomedon* cf. *massiliensis Bellan-Santini 1965

Hippomedon oculatus Chevreux & Fage 1925

Genus *Lepidepecreum* Bate & Westwood 1868

Lepidepecreum subclypeatum *R*uffo & Schiecke 1977

Genus *Lysianassa* Milne-Edwards 1830

Lysianassa* cf. *plumosa Boeck 1871

Genus *Normanion* Bonnier 1893

Normanion* cf. *ruffoi Diviacco & Vader, 1988

Genus *Paracentromedon* Chevreux & Fage 1925

Paracentromedon crenulatum Chevreux 1900

Genus *Perrierella* Chevreux & Bouvier 1892

Perrierella audouiniana (Bate 1857)

Family Melitidae Bousfield 1973

Genus *Ceradocus* Costa, 1853

Ceradocus semiserratus (Bate 1862)

Genus *Eriopisa* Wrzesniovsky 1890

Eriopisa elongata (Bruzelius 1859)

Family Melphidippidae Stebbing 1899

Genus Melphidippella Sars 1894

Melphidippella macra (Norman 1869)

Family Oedicerotidae Lilljeborg 1865

Genus *Bathymedon* Sars 1892

Bathymedon acutifrons Bonnier 1896

Bathymedon sausseri (Boeck, 1871)

Genus *Halicreion* Boeck 1871

***Halicreion* sp.**

Genus *Monoculodes* Stimpson 1853

Monoculodes acutipes Ledoyer 1983

Monoculodes packardi Boeck 1871

Monoculodes subnudus Boeck 1872

Genus *Oediceroides* Stebbing 1888

Oediceroides pilosa Ledoyer 1983

Genus *Perioculodes* Sars 1895

Perioculodes* cf. *aequimanus (Korssman 1880)

Perioculodes longimanus (Bate & Westwood 1868)

Genus *Synchelidium* Sars 1892

Synchelidium cf. longidigitatum Ruffo 1947

Synchelidium maculatum Stebbing 1906

Genus *Westwoodilla* Bate 1862

Westwoodilla cf. caecula (Bate 1857)

Family Pardaliscidae Boeck 1871

Genus *Halice* Boeck 1871

Halice cf. abyssi Boeck 1871

Genus *Nicippe* Bruzellius 1859

Nicippe tumida Bruzelius 1859

Family Phoxocephalidae Sars 1891

Genus *Harpinia* Boeck 1876

***Harpinia* sp.**

Harpinia* cf. *antennaria Meinert 1890

***Harpinia* crenulata** (Boeck 1871)

Harpinia* cf. *pectinata Sars 1891

Harpinia truncata Sars, 1891

Genus *Leptophoxus* Sars 1891

Leptophoxus falcatus (Sars 1883)

Genus *Metaphoxus* Bonnier 1896

Metaphoxus simplex (Bate 1857)

Family Sebidae Walker 1908

Genus *Seba* Bate 1862

Seba aloe Karaman 1971

Family Stegocephalidae Dana 1855

Undetermined

Family Stenothoidae Boeck 1871

Genus *Metopa* Boeck 1871

***Metopa* sp.**

Genus *Stenothoe* Dana 1852

***Stenothoe* sp.**

Stenothoe eduardi Krapp-Schickel 1975

Stenothoe cf. marina (Bate 1856)

Stenothoe valida Dana 1852

Family Synopiidae Dana 1853

Genus *Bruzelia* Böck 1871

Bruzelia typica Boeck 1871

Genus *Pseudotiron* Chevreux 1895

Pseudotiron bouvieri Chevreux 1895

Genus *Syrrhoe* Chevreux 1908

Syrrhoe cf. affinis Chevreux 1908

Syrrhoe* cf. *angulipes Ledoyer 1977

Family Urothoidae Bousfield 1978

Genus *Carangolia* J.L. Barnard 1961ª

Carangolia barnadi Jaume & Sorbe, 2001

Family Valettiidae Stebbing 1888

Genus *Valettietta* Lincoln & Thurston 1983

***Valettietta* sp.**

Suborder Hyperiidae Milne Edwards 1830

Family Hyperiidae H. Milne Edwards 1830

Genus *Themisto* Guérin-Ménéville 1828

Themisto gaudichaudi Guérin, 1825

Family Phrosinidae Dana 1853

Genus *Primno*

Primno macropa Guérin-Méneville 1836

Order Cumacea

Family Bodotriidae T. Scott 1901

Genus *Eocuma* Marcusen 1894

***Eocuma* sp.**

Family Ceratocumatidae Calman 1905

Undetermined

Family Diastylidae Bate 1856

Undetermined

Family Lampropidae Sars 1878

Undetermined

Family Leuconidae Sars 1878

Genus *Eudorella* Norman 1867

Eudorella emarginata (Krøyer 1846)

Genus *Leucon* Krøyer 1846

***Leucon* spp.**

Family Nannascitidae Bate 1866

Undetermined

Genus *Campylaspis* G.O. Sars 1865

***Campylaspis* sp**. (7 species)

Campylaspis cf. horrida Sars 1870

Genus *Cumella* G.O. Sars 1865

***Cumella* sp**.

Genus *Procampylaspis* Bonnier 1896

***Procampylaspis* sp**.

Order Isopoda Latreille 1817

Suborder Asellota Latreille 1802

Family Acanthaspidiidae Menzies 1962

Genus *Mexicope* Hooker 1985

Cf. *Mexicope* sp.

Family Dendrotionidae Vanhoeffen 1914

Genus *Dendrotion* G.O. Sars 1872

Dendrotion cf. elegans Lincoln & Boxshall 1983

Family Desmosomatidae G. O. Sars 1897

Undetermined

Genus *Chelator* Hessler 1970

Chelator spp.

Chelator cf. insignis (Hansen 1916)

Chelator cf. verecundus Hessler 1970

Genus *Eugerda* Meinert 1890

Eugerda cf. fulcimandibulata Hessler 1970

Genus *Eugerdella* Kussakin 1965

Eugerdella cf. ischnomesoides Hessler 1970

Eugerdella cf. pugilator Hessler 1970

Genus *Mirabilicoxa* Hessler 1970

Mirabilicoxa cf. acuminata Hessler 1970

Genus *Prochelator* Hessler 1970

Prochelator sp.

Prochelator lateralis (G. O. Sars 1899)

Family Haploniscidae Hansen 1916

Genus *Haploniscus* Richardson 1909

***Haploniscus* sp**.

Haploniscus cf. angustus Lincoln 1985

Family Ischnomesidae Hansen 1916

Undetermined

Genus *Haplomesus* Richardson 1908

***Haplomesus* sp**.

Genus *Ischnomesus* Richardson 1908

Ischnomesus sp.

Family Janirellidae Menzies 1956

Genus *Janirella* Menzies 1956A

***Janirella* sp.**

Janirella cf. hessleri Chardy 1975

Janirella cf. nanseni Bonnier 1896

Family Janiridae Sars 1897

Genus *Austrofilius* Hodgson 1910

***Austrofilius* sp.**

Genus *Janira* Leach 1814

Janira maculosa Leach, 1814

Family Joeropsididae Nordenstam 1933

Undetermined

Family Katianiridae Svavarsson 1987

Genus *Katianira* Hansen 1916

***Katianira* sp**.

Family Macrostylidae Hansen 1916

Genus *Macrostylis* Sars 1864

***Macrostylis* sp**.

Macrostylis magnifica Wolff 1962

Family Munnidae Sars 1897

Genus *Munna* Krøyer 1839

***Munna* sp.**

Family Munnopsidae Lilljeborg 1864

Genus *Aspidarachna* Sars 1899

Aspidarachna cf. clypeata Sars 1899

Genus *Disconectes* Wilson & Hessler 1981

***Disconectes* spp**.

Genus *Eurycope* Sars 1864

Eurycope complanata complex

Genus *Ilyarachna* Sars 1870

***Ilyarachna* spp.**

Genus *Munneurycope* Stephensen 1912

Munneurycope cf. nodifrons (Hansen 1916)

Genus *Storthyngura* Vanhöffen 1914

***Storthyngura* sp.**

Genus *Syneurycope* Hansen 1916

***Syneurycope* sp.**

Family Nannoniscidae Hansen 1916

Genus *Hebefustis* Siebenaller & Hessler 1977

***Hebefustis* sp.**

Genus *Nannoniscoides* Hansen 1916

***Nannoniscoides* sp.**

Genus *Nannoniscus* G. O. Sars 1870

***Nannoniscus* sp.**

Family Paramunnidae Vanhöffen 1914

Genus *Notoxenoides* Menzies 1962

***Notoxenoides* sp.**

Genus *Paramunna* G.O. Sars 1866

Paramunna bilobata G.O. Sars 1866

Genus *Pleurogonium* G.O. Sars 1864

Pleurogonium aff. pulchrum Hansen 1916

Family Thambematidae Stebbing 1913

Genus *Thambema*

***Thambema* sp.**

Suborder Cymothoida Wägele 1989

Family Cirolanidae Dana 1852

Genus *Eurydice* Leach 1815

Cf. *Eurydice* sp.

Genus *Metacirolana* Nierstrasz 1931

Metacirolana cf. hanseni (Bonnier 1896)

Genus *Natatolana* Bruce 1981

***Natatolana* sp.**

Family Gnathiidae Leach 1814

Genus *Gnathia* Leach 1814

***Gnathia* spp.**

Genus *Monodgnathia* Cohen & Poore 1994

Monodgnathia cristatipes (Stebbing 1912)

Family Hyssuridae Wägele 1981

Undetermined

Genus *Hyssura* Norman & Stebbing 1886

***Hyssura* sp.**

Family Leptanthuridae Poore 2001

Genus *Leptanthura* Sars 1897

***Leptanthura* sp.**

Family Paranthuridae Menzies & Glynn 1968

Genus *Paranthura* Bate & Westwood 1866

***Paranthura* sp.**

… Suborder Valvifera Sars 1882

Family Arcturidae Dana 1849

Genus *Astacilla* Cordiner 1793

***Astacilla* spp**. (2 species)

Order Lophogastrida

Undetermined

Genus *Gnathophausia* Willemoes-Suhm 1873

Gnathophausia zoea Willemoes-Suhm 1875

Order Tanaidacea Dana 1849

Family Anarthruridae Lang 1971

Undetermined (3 species)

Genus *Haplocope* Sars 1882

***Haplocope* sp.**

Family Apseudidae Leach 1814

Undetermined (5 species)

Genus *Apseudes* Leach 1814

Apseudes grossimanus Norman & Stebbing 1886

Family Colletteidae Larsen & Wilson 2002

Genus *Collettea* Lang 1973

***Collettea* sp.**

Genus *Isopodidus* Larsen & Heard 2002

***Isopodidus* sp.**

Genus *Leptognathiella* Hansen 1913

***Leptognathiella* sp.**

Family Leptocheliidae Lang 1973

Genus *Bathyleptochelia* Larsen 2003

Cf. *Bathyleptochelia* sp.

Family Leptognathiidae Lang 1976

Genus *Leptognathia* Sars 1882

***Leptognathia* spp** (3 species)

Family Nototanaidae Sieg 1976

Genus *Paratyphlotanais* Kudinova-Pasternak & Pasternak, 1978

***Paratyphlotanais* sp.**

Family Pseudotanaidae Sieg 1976

Undetermined

Genus *Pseudotanais* Sars 1882

***Pseudotanais* sp.**

Family Sphyrapidae Gutu 1980

Undetermined

Genus *Sphyrapus* Sars 1882

Sphyrapus malleolus Norman & Stebbing 1886

Family Tanaellidae Larsen and Wilson 2002

Genus *Araphura* Bird & Holdich 1984

***Araphura* sp.**

Genus *Tanaella* Norman & Stebbing 1886

Tanaella cf. unguicillata Norman & Stebbing 1886

Family Typhlotanaidae Sieg 1986

Genus *Obesutanais* Larsen et al. 2006

***Obesutanais* sp.**

Genus *Typhlotanais* Sars 1882

***Typhlotanais* spp.** (4 species)

Paratanaoidea incertae sedis

Genus *Coalecerotanais* Larsen 2003

***Coalecerotanais* sp.**

Genus *Spinitanaopsis* Larsen 2005

***Spinitanaopsis* sp.**

Phyllum ECHINODERMATA

Class Asteroidea de Blainville 1830

Undertermined

Class Crinoidea

Suborder Comatulidina A.H. Clark 1908

Family Antedonidae Norman 1865

Genus *Antedon* de Freminville 1811

***Antedon* sp.**

Class Echinoidea

Order Cidaroida

Family Cidaridae

Undetermined

Order Spatangoida

Family Brissidae

Genus *Brissopsis* L. Agassiz, in L. Agassiz & Desor 1847

Brissopsis lyrifera (Forbes 1841)

Class Stelleroidea Lamarck 1816

Subclass Ophiuroidea Gray 1840

Order Ophiurida Müller & Troschel 1840

Family Ophiacanthidae Perrier 1891

Genus *Ophiacantha* Koehler 1911

Ophiacantha aristata Koehler 1896

Genus *Ophiopristis* Verril 1899

Ophiopristis cadiza sp. nov. Rodrigues *et al*. 2009

Family Ophiactidae Matsumoto 1915

Genus *Ophiactis* Lütken 1856

Ophiactis abyssicola (M. Sars, 1861)

Ophiactis balli (Thompson, 1840)

Family Amphiuridae Ljungman 1867

Genus *Amphiura* Forbes 1843

Amphiura sp. sensu Storh & Segonzac 2005

Amphiura grandisquama Lyman 1869

Amphiura chiajei Forbes 1843

Amphiura filiformis (O.F. Müller 1776)

Genus *Amphioplus* Verrill 1899

Amphioplus hexabrachiatus *Stohr 2003*

Genus *Amphipholis* Thomas 1966

Amphipholis squamata (Delle Chiaje 1829)

Genus *Amphilepis* Ljungman 1867

Amphilepis ingolfiana Mortensen 1933

Family Ophiuridae Müller & Troschel 1840

Genus *Ophiura* Lamarck 1801

Ophiura (Dictenophiura) carnea Lütken 1858

Genus *Ophiomusium* Lyman 1869

Ophiomusium lymani W. Thomson 1873

This list was compiled in collaboration with several specialists:

Blazewicz-Paszkowycz, Magda – University of Lodz (Crustacea: Tanaidacea)

Cunha, Marina R. – CESAM, Universidade de Aveiro (Polychaeta: Siboglinidae)

Dworschak, Peter C. – Naturhistorisches Museum, Wien (Crustacea: Callianassidae)

Génio, Luciana – CESAM, Universidade de Aveiro (Mollusca: Mytilidae)

Hilário, Ana – CESAM, Universidade de Aveiro (Polychaeta: Siboglinidae)

Lopèz-Gonzaléz, Pablo – Universidade de Sevilla (Cnidaria: Anthozoa)

Moura, Carlos J. – CESAM, Universidade de Aveiro (Cnidaria: Hydrozoa)
Oliver, Graham – National Museum of Wales (Bivalvia)
Paterson, Gordon – The Natural History Museum, London (Echinodermata: Ophiuroida)
Ravara, Ascensão – CESAM, Universidade de Aveiro (Polychaeta)
Rivellaud, Julie – Ghent University (Porifera)
Rodrigues, Clara F. – CESAM, Universidade de Aveiro (Bivalvia, Echinodermata: Ophiuroida)
Sorbe, Jean-Claude – CNRS, Arcachon (Crustacea)
van Soest, Rob – Zoological Museum of Amsterdam (Porifera)
Wilson, George – Australian Museum, Sidney (Crustacea: Asellota)